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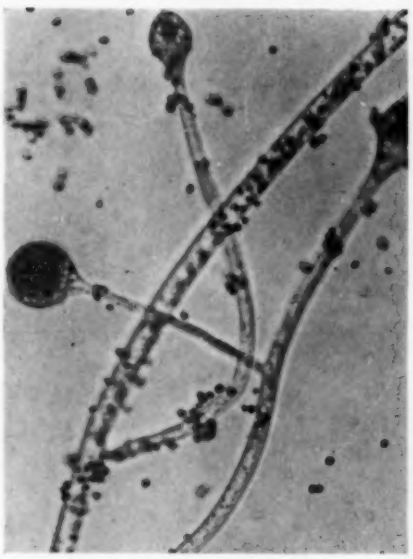

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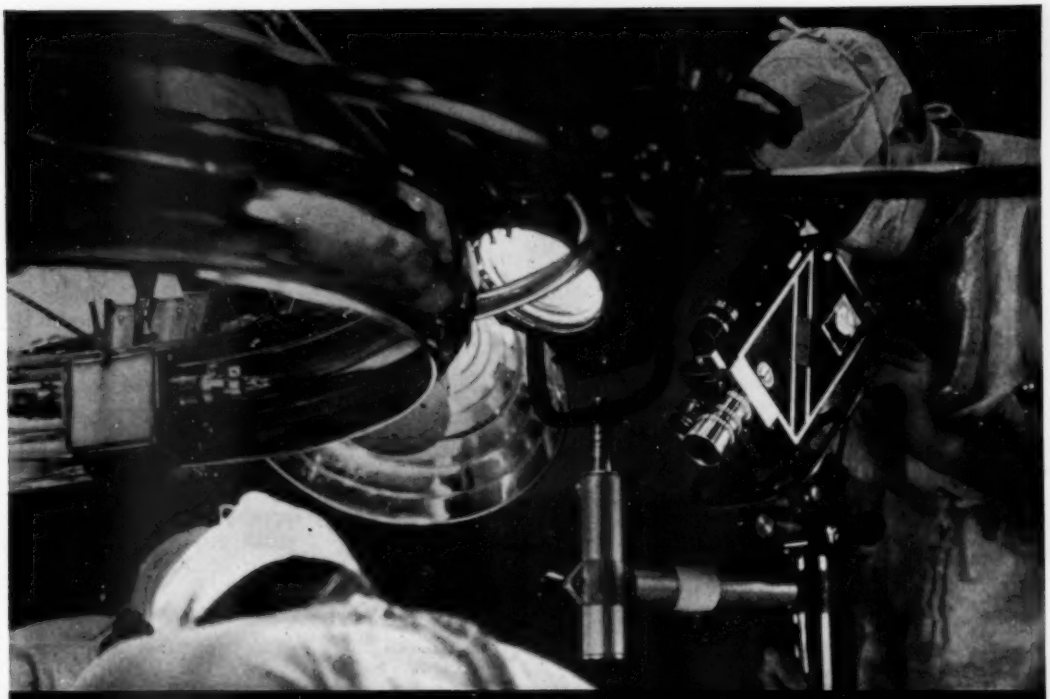

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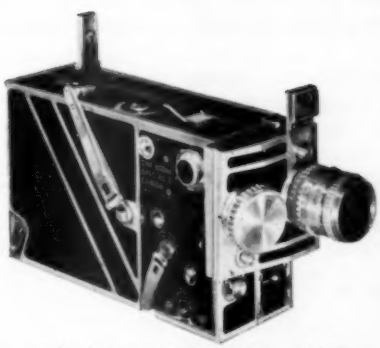

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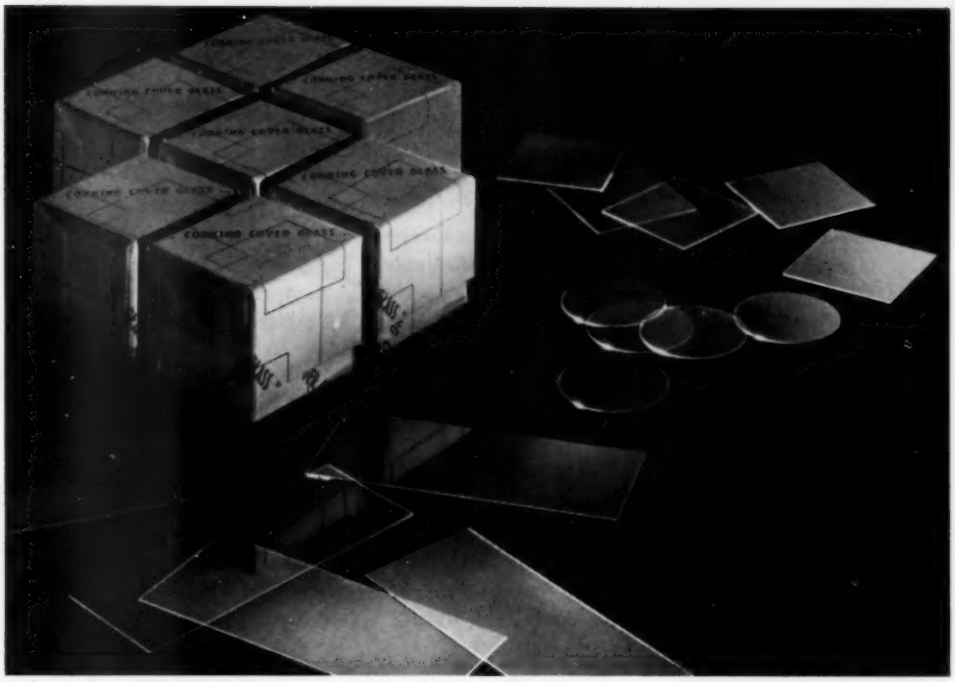

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A.M.A. Archives OF PATHOLOGY

Cirrhosis of the Liver in Sickle Cell Disease

Report of a Case with a Review of the Literature

YO SEUP SONG, M.D., Memphis

The first recognition of sickle-cell disease is credited to Herrick,[1] who in 1910 first described a case of severe anemia. Washburn [2] described a similar case the following year. Cook and Meyer [3] reported the third case in 1915, and Mason [4] described a fourth case in 1922. In 1923 Sydenstricker, Mulherin, and Houseal [5] described two cases in detail and reported collateral study on 12 relatives, 9 of whom were found to show evidences of sickle-cell phenomenon. In one of their cases the necropsy findings were reported, making the first postmortem study of this disease on record. Finally, Sydenstricker [6] summarized the knowledge of the disease and reported a second autopsy case. Thereafter, numerous authors in additional necropsy reports have reviewed the pathological lesions produced by sickle-cell phenomena. In 1928 Rich [7] reviewed, of 5000 consecutive autopsies, 62 cases of sickle cell disease in which the livers were consistently enlarged. In one case cirrhosis was present, but this was thought to be a coincidence, as there were no characteristic lesions found in other cases coming to autopsy. Since this description was made, relatively less attention has been paid to the hepatic lesions in comparison with those changes in the spleen or bone marrow about which detailed descriptions were made repeatedly.

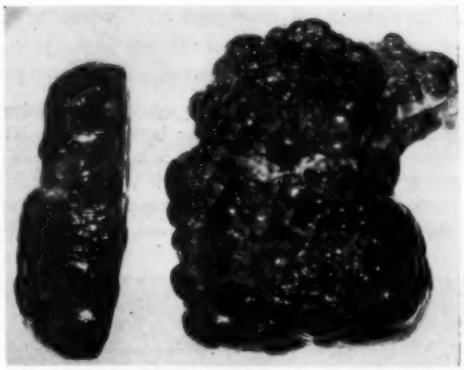


Fig. 1.—Cirrhotic liver showing marked variation in size of nodules which have deep-red color; enlarged spleen showing numerous discrete nodules which are bulging.

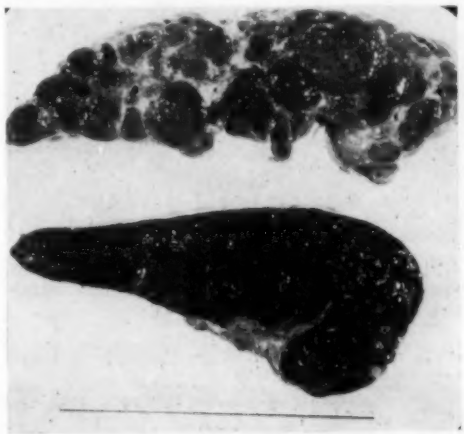


Fig. 2.—Varying-sized hepatic nodules are separated by broad gray fibrillary tissue.

Recently, I have had the opportunity to study a case of hepatic cirrhosis in sickle cell

Submitted for publication May 21, 1955.

Division of Pathology and Microbiology, University of Tennessee and City of Memphis Hospitals.

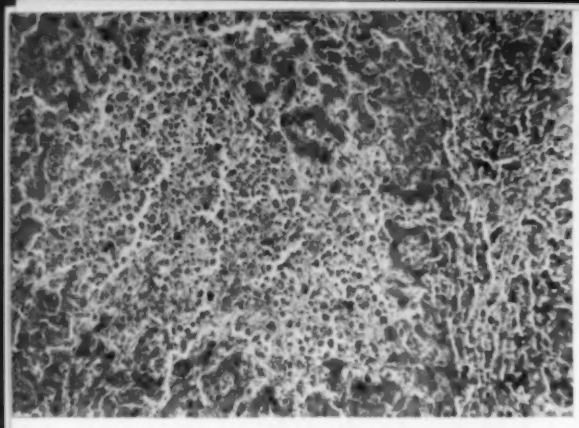


Fig. 3.—Large zone of cellular necrosis.

disease. This case, with a review of literature, is the subject of this report.

CASE REPORT

H. P., a 15-year-old Negro boy, was readmitted to the City of Memphis Hospital because of massive hematemesis with circulatory collapse. Approximately seven years prior to this he was treated for pneumococcic meningitis and responded well to penicillin and sulfadiazine. On that admission he was anemic, with positive sickle cell preparation. The liver and spleen were enlarged and firm. Repeated blood transfusions failed to raise the hemoglobin level above 8.0 gm. per 100 cc. The bleeding time, clotting time, and platelet count were, however, normal. Following recovery from the meningitis, examination of the blood revealed a leucocyte count of 7250 (12% band forms, 55% segmented forms, 1% eosinophiles, 1% basophiles, 29% lymphocytes) and 2 nucleated red cells.

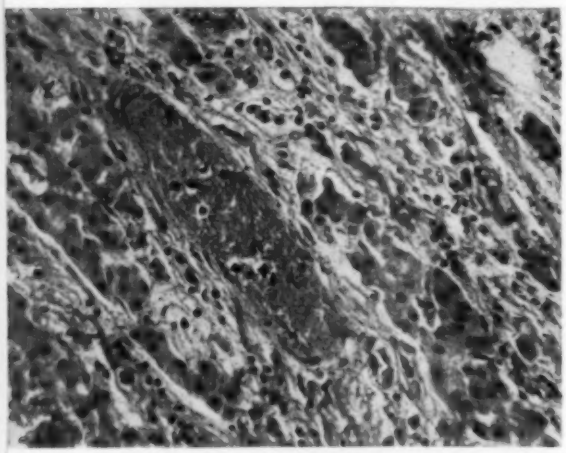
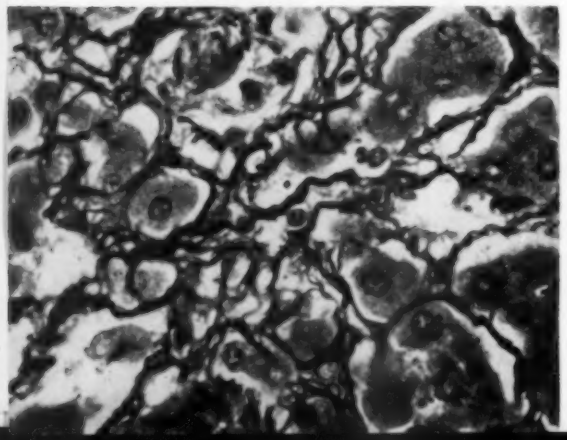


Fig. 4.—The vessel is obliterated by numerous sickled red blood cells. Note the marked dissociation of hepatic cells.

Fig. 5.—Reticulum fibers are obviously thickened.



On the second admission the patient was in profound shock, without detectable blood pressure, respiration, or heart sounds. Before being brought to the hospital, he vomited blood twice in large quantities. The lymph nodes were generally enlarged, being firm, discrete, and movable. Lungs and heart could not be examined adequately. The liver was palpable about 3 cm. below the costal margin at the level of the umbilicus and was questionably nodular. The hematocrit reading was less than 10 mm., and the hemoglobin measured less than 2.5 gm. White blood cell count was 40,000 with predominant segmented neutrophiles. After the administration of intravenous fluid, epinephrine, caffeine, oxygen, and plasma and whole blood, the blood pressure rose to a maximum of 96/50. The heart rate was 72, and the respirations were occasional and irregular. Despite the treatment the response was transitory, and the patient died after six hours in the hospital.

At postmortem examination the body was well nourished and well developed. Icterus was obvious. The extremities and external surfaces showed no evidence of ulceration. The peritoneal and pleural cavities were free from exudate or transudates.

The heart was not unusual, showing no evidence of inflammation or vascular lesions. The liver was not increased in weight but was markedly distorted, owing to varying-sized nodules in it. These nodules were separated by a grayish, firm fibrillary tissue and varied up to 4 cm. in diameter (Fig. 1). The bile ducts and gall bladder were not unusual. The spleen was markedly enlarged, weighing 900 gm., and consisted of firm, grayish-pink tissue in which numerous discrete nodules of bulging, reddish-black tissue were seen (Fig. 2). Both lungs were heavy and markedly hemorrhagic. The kidneys and adrenals were extremely congested. In the lower portion of the esophagus were varicosities which undoubtedly had caused the hematemesis and gastrointestinal hemorrhage.

On microscopic examination of the liver the lobular patterns were generally irregular or absent. Occasionally a patchy type of rather fresh cellular necrosis was seen. Surrounding this necrotic zone, the sinusoids were dilated and stuffed with sickle cells (Fig. 3). In addition to these, the hepatic

arteries were obliterated by an agglutinative material composed of numerous sickle red cells (Fig. 4). Around these, the hepatic cells were markedly dissociated. Generally the sinusoids were tremendously dilated and stuffed with sickle red cells. The reticulum fibers were thickened and collapsed upon one another (Fig. 5). There were scanty amounts of iron pigment noted in the liver cells, but the scar tissue was free from pigmentation.

The Kupffer cells were not particularly swollen. There was no bile duct proliferation. Around the portal space the fibrous elements were markedly hyperplastic, separating the hepatic lobules and forming pseudolobules. Heavy round cell infiltration was seen throughout the entire section, but was severest in the portal space.

Under the slightly thickened splenic capsule a few fairly large, well-circumscribed hemorrhagic areas were present. In between these hemorrhagic areas there was an atrophic white pulp.

A moderate amount of iron pigment was seen throughout the hemorrhagic tissue. No evidence of fibrosis was seen.

The renal vessels were extremely congested, particularly the glomerular capillaries, which were filled with somewhat hyaline-like material composed of sickle cells. The tubular epithelium contained small amounts of iron pigment.

The alveoli of the two lungs were filled with massive amounts of blood and edema fluid. The pulmonary arterioles and alveolar capillaries were actually obliterated by numerous sickled red blood cells. A few of the arterioles were of interest, showing marked proliferation of the intimal cells suggestive of recanalization. A few of the sickle cells were still identifiable in thrombosed vessels (Fig. 6).

The submucosal blood vessels of the lower esophagus were greatly dilated and surrounded by many polymorphonuclear neutrophiles. The gastrointestinal tract showed nothing unusual. The brain was markedly edematous, containing numerous dilated blood

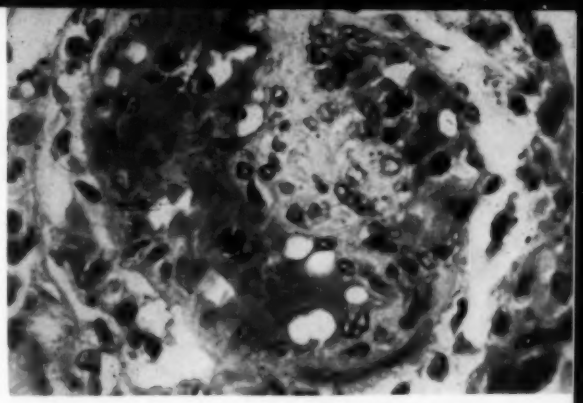


Fig. 6.—Pulmonary arteriole showing recanalizing process.

vessels in which definite agglutinative clots were noted. The lymph nodes were hyperplastic, without showing any neoplastic or inflammatory changes.

Sections of bone marrow revealed a moderate degree of ischemic necrosis of the trabeculae; the marrow was extremely cellular, being markedly hyperplastic with numerous sickle cells.

REVIEW OF LITERATURE

The lesions of the liver produced by the sickle-cell phenomena seem to be variable. As a rule, the liver is consistently moderately enlarged, showing various degrees of regressive changes. Corrigan and Schiller [8] believed that the liver is enlarged and varies in color from deep purple to brown.

Lowe and Adams [9] suggested that the hepatomegaly is probably due to stagnation of sickle cells in the hepatic sinusoids. Rigdon [10] mentioned that the enlarged liver, which is deep purple, contains portal capillaries engorged with sickle cells.

Ryerson and Terplan [11] presented two postmortem cases in detail. The extrahepatic ducts were not unusual, but the lobules were obscured by marked capillary dilatation. Severe regressive changes were observed. The individual hepatic cells were moderately degenerated, this varying from fatty droplet degeneration to loss of nuclei and complete necrosis.

Heilbrun [12] and Hamman [13] noted marked atrophy of the liver cell cords with pigmentation, extreme dilatation of sinusoids, and round cell infiltration around the portal spaces.

Sydenstricker, Mulherin, and Houseal [5] and Page and Silton [14] reported similar changes. Jaffé,[15] on the other hand, described the Kupffer cells as being generally swollen and showing prominent granules and phagocytosis of sickled red blood cells.

Steinberg [16] in 1930 made a detailed description of the lesions in the lung, spleen, and liver. In his seven cases the liver showed increased connective tissue in the central and paracentral lobular zones, as well as an increase in the periportal connective tissue.

Ching and Diggs [17] described agglutinative thrombi in hepatic capillaries around which heavy cell infiltration was present. Crastnopol and Stewart [18] described the liver biopsy in sickle-cell patients. The hepatic capillaries were occluded by agglutinative thrombi, and the lesions were sufficient to establish a diagnosis of chronic hepatitis.

Graham [19] presented one case of chronic hepatitis which showed tremendous cellular degeneration and fine patchy irregularly distributed lymphocytic infiltrations which were noted particularly around the portal spaces. Similar changes were observed by Ryerson and Terplan [11] and Dale,[20] who suggested the term subacute toxic dystrophy or subacute liver atrophy for those lesions noted in sickle cell deaths. Simultaneously, Tomlinson [21] reported one case of severe hemosiderosis associated with marked fibrosis.

Hargrove and Matthews [22] in 1940 presented a case of diffuse hemochromatosis which was very similar to the case reported by Tomlinson. The cells were extremely pigmented, and diffuse fibrosis was present. Round cell infiltration was seen throughout the tissue.

Kimmelstiel [23] described the hepatic lesion in a postmortem case of an 11-year-old Negro girl. The liver showed many areas of necrosis surrounded by narrow hemorrhagic zones. However, no vascular thrombi were noted.

Shortly after this, Legant and Ball [24] reported a case of liver cirrhosis in a sickle cell death. Four other cases showed severely damaged livers with extensive cellular degeneration and massive necrosis of hepatic cells. None of these cases had a history of hepatitis or of other infectious diseases.

Green, Conley, and Berthrong [25] in 1953 made an extensive study in 21 cases of sickle-cell patients, in which four instances of liver cirrhosis were present.

SUMMARY AND COMMENT

A postmortem study of the pathologic findings in a patient dying in the course of sickle-cell anemia is presented. The patient was a 15-year-old Negro boy who had been known to have sickle cell anemia for at least eight years. On the first hospital admission he had a nodular liver with an enlarged spleen. There was no history of hepatitis or syphilis.

The splenic lesions are similar to those observed by Sullivan [26] and by Cooley, Peterson, Engle, and Jernigan,[27] who described splenic enlargement and infarction in sickle-cell patients as a result of airplane flights. In those patients the spleen regularly enlarged approximately three times normal size and grossly was infarcted. The largest spleen weighed 804 gm. They concluded that the massive splenic infarction in sickle-cell patients is probably due to low oxygen tension followed by high altitude flight.

Cirrhosis of the liver in the present case was thought to be of a particular type, showing varying lesions from fresh necrosis to marked fibrosis forming the pseudolobules with round cell infiltration. The agglutinative thrombi of the hepatic capillaries and the sinusoidal obstruction produced by sickle cells are probably the cause of cellular necroses with subsequent fibrosis. The gross appearance of the liver as illustrated in Figure 1 is certainly suggestive of the type of cirrhosis as being postnecrotic cirrhosis.

The lesions of the liver described in this case are presumably a manifestation of sickle cell disease.

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Studies on Mixed Infections

II. Pathological Effects of Combined Brucella Suis and Coxiella Burnetii Infection

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Combined infection with Brucella suis and Coxiella burnetii produced a milder illness than infection with the same dose of agents administered separately. The severity of the disease was estimated from changes in blood cell counts, temperature, weight, and serological reactions.[1] The pathological effects of combined and separate infection with B. suis and C. burnetii in the guinea pigs described in the report cited above are the subject of the present study.

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METHOD

Guinea pigs weighing 300 to 350 gm. were exposed to an aerosol dose of either 5000 B. suis cells or 86 guinea pig ID_{50} of C. burnetii, or both, in the Reyniers chamber.[7] The combined infection was produced by administering the brucellae immediately before the Coxiella.[1] At intervals varying from four days to eight months thereafter, two guinea pigs of each of the three groups were bled and then killed by chloroform inhalation. All tissues were fixed in Zenker's solution and stained with Lillie's modification of the Giemsa stain.

RESULTS

Tissue alterations are summarized in the Tables according to the interval after exposure. Lymph node and spleen changes are recorded as sizes relative to normal and the dominant histological character of the lesions is noted in Tables 1 to 3. Other symbols are defined in the footnotes to the Tables.

TABLE 1.—*Gross and Microscopic Lesions in Brucellosis in Guinea Pigs* *

Days	Lymph Nodes				Spleen	Lung	Liver	Renal Pelvis	Bone Marrow	Lacrimal Gland
	Submentel	Cervical	Inguinal	Tracheal						
4	..	..	..	..	..	..	..	..	..	..
4	..	..	..	..	..	..	..	..	..	..
10	..	..	..	RE	..	..	FN	..	..	..
10	..	..	..	..	..	..	..	..	..	..
14	..	3G	..	3G	RE	1	..	..	<	..
14	2RE	3RE	..	3RE	RE	1	FN	..	..	..
17	1G	3G	RE	4G	3G	2	FN	..	..	I
17	2G	4G	2G	4G	3G	3	G	G	G	G
21	2RE	2G	3RE	5G	4G	2	G	G	G	..
21	3RE	8G	3RE	8G	4G	1	G	G	G	..
27	4RE	10G	3RE	5G	3G	±	G	G	G	..
27	4RE	6G	3RE	3G	4G	±	G	G	G	..
41	3G	6G	3G	6G	8G	1	G	G	G	G
41	4G	6G	4G	3G	6G	1	G	G	G	..
56	1G	2G	4G	4G	4G	..	..	G	G	G
56	3G	3G	2G	6G	4RE	±	FN	G	G	I
118	..	F	2F	2F	F	..	FN	..	..	I
118	2A	4A	2A	3A	4A	1	4A	..	..	..

* Numerals indicate relative size of lesions; for lung they show number of pneumonic lobes. RE, hyperplasia of reticuloendothelial cells; G, granuloma; FN, focal necrosis; A, abscess; F, fibrosis; M, basophilic mononuclear cells; I, leucocyte infiltration; P, pigment; <, decreased neutrophiles; >, increased neutrophiles.

Gross Changes in Brucellosis Due to B. Suis

Gross lesions occurred in the lymph nodes, spleen, liver, and lungs (Table 1). Lymph nodes were enlarged up to 10 to 15 times, showing varying degrees of induration, caseation, and abscess formation. These changes began in the tracheal and cervical lymph nodes but later occurred in all the nodes. Spleens measured up to six times the usual width and thickness and three times the usual length; had nodules of necrotic or caseous white or yellow tissue, fibrous adhesions, or diffuse induration, and returned to normal size as the lesions healed. Lungs had small patches of red consolidation during the third week after exposure. The liver showed tiny white and yellow nodules which did not protrude through the capsule, varying from just visible specks to 1 mm. in diameter. At four and eight months fibrosis and abscesses were the most conspicuous changes involving various lymph nodes, spleen, and liver.

Gross Lesions in Q Fever

Gross lesions after aerosol exposure to C. burnetii appeared in the lungs within four days and persisted for three weeks, decreasing in extent after two weeks (Table 2). Nodular red consolidation was confined to two lobes or less. The pleura and cut surfaces were wet, shiny, red, and opaque. Spleens and cervical and bronchial lymph nodes were slightly enlarged between two and three weeks after infection. These enlarged lymph nodes had considerable pericapsular scarring. No gross lesions attributable to Q fever occurred after four weeks.

Gross Lesions in Combined Brucellosis and Q Fever

Combined exposure to B. suis and C. burnetii produced less lymph node enlargement and pulmonary consolidation than B. suis alone (Tables 1, 3, and 4). However, scattered areas of minute red pulmonary consolidation resembling those of Q fever occurred as early as four days. Lymph node abscesses and fibrosis appeared earlier in the combined infection, where they were first noted at 6 weeks instead of at 15 weeks as in brucellosis alone. Otherwise, gross lesions of brucellosis were similar in mixed and separate infections.

Histological Lesions in Guinea Pigs Exposed to Aerosols Containing B. Suis

After aerosol exposure to B. suis, the earliest microscopic lesions were found at 10 days in the lungs and tracheal lymph nodes (Table 1). Although not enlarged, the latter showed diffusely distributed large pale-staining mononuclear cells occasionally forming granulomata containing polymorphonuclear leucocytes and mononuclear cells with oval or round nuclei and deeply basophilic cytoplasm (Fig. 1). The capsule was free of reaction. Pneumonic areas less than 1 mm. in diameter showed exudate of mononuclear cells and fibrin in alveoli and thickening of alveolar walls by mononuclear cells (Fig. 2). The bronchi were not infiltrated. At 14 days pulmonary lesions were grossly evident and the histological reaction involved bronchi as well as alveoli (Fig. 3). Cervical as well as tracheal lymph nodes contained granulomata. Occasionally caseation and necrosis in granulomata of the cervical lymph nodes indicated that these lesions were older than those in tracheal lymph nodes which showed granulomata without necrosis. In a few animals granulomata occurred in only one cervical and/or tracheal lymph node, without pulmonary lesions, although splenic hyperplasia of reticulum cells (Fig. 4) and focal necrosis of the liver were present. At 17 days granulomata appeared around ducts and in the acinar tissue of the Harderian gland in the retro-orbital tissue (Figs. 5 and 6), a novel finding in brucellosis.

Generalized granulomatosis was noted by three weeks, with lesions in the myocardium, endocardium (Fig. 7), epicardium, lungs, spleen (Fig. 8), liver, lymph nodes, renal pelvis (Fig. 9), epididymis, uterus, and bone marrow; but the most advanced lesions were in the cervical and/or tracheal lymph nodes (Fig. 10), showing caseous necrosis, whereas granulomata elsewhere had no necrosis. At six weeks the primary lesion was not readily detectable because the secondary foci were

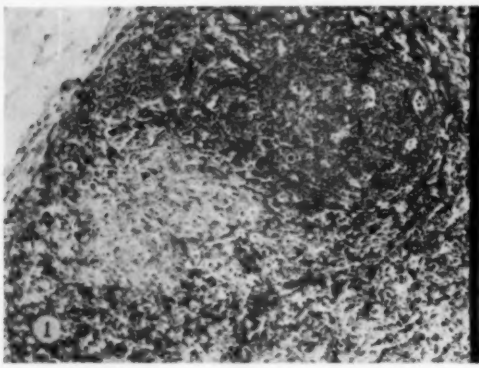


Fig. 1.—Brucellosis 10 days. Tracheal lymph nodes. Small nodules of epithelioid cells in pulp with diffuse proliferation of similar cells. Capsule free of inflammatory cells. Giemsa stain; reduced about ⅙ from mag. × 180.

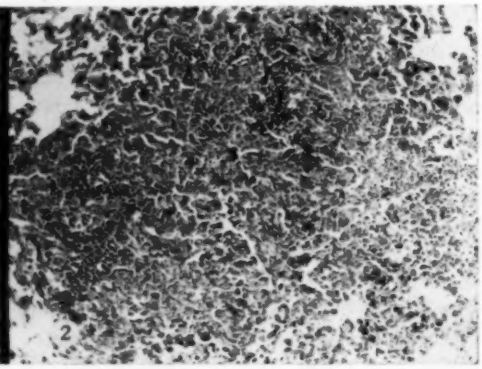


Fig. 2.—Brucellosis 10 days. Focal interstitial pneumonia. Mononuclear cells and fibrin in alveolar spaces. Infiltration of alveolar septa. Giemsa stain; reduced about ⅙ from mag. × 180.

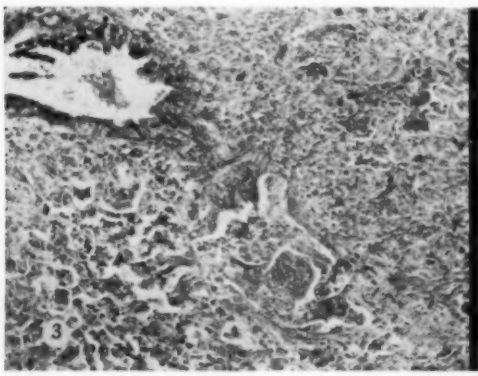


Fig. 3.—Brucellosis 14 days. Interstitial pneumonia. Bronchial and alveolar exudate of mononuclear cells and fibrin in walls and spaces. Giemsa stain; reduced about ⅙ from mag. × 180.

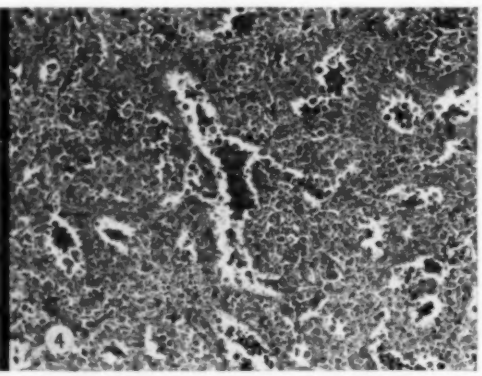
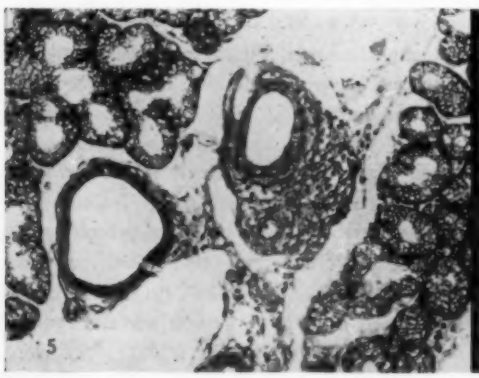


Fig. 4.—Brucellosis 14 days. Spleen. Hyperplasia of reticulum cells widening pulp cords. Giemsa stain; reduced about ⅙ from mag. × 180.

Fig. 5.—Brucellosis 17 days. Harderian gland. Granuloma of epithelioid cells around duct. Giemsa stain; reduced about ⅙ from mag. × 180.

Fig. 6.—Brucellosis 17 days. Harderian gland. Epithelioid and lymphoid cells and necrosis of acini. Giemsa stain; reduced about ⅙ from mag. × 180.



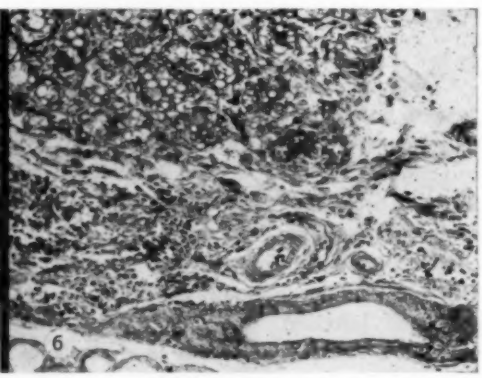


Fig. 7.—Brucellosis 21 days. Heart. Epithelioid and mononuclear cell proliferation and endothelial swelling in endocardium of left ventricle. Giemsa stain; reduced about ⅕ from mag. × 180.

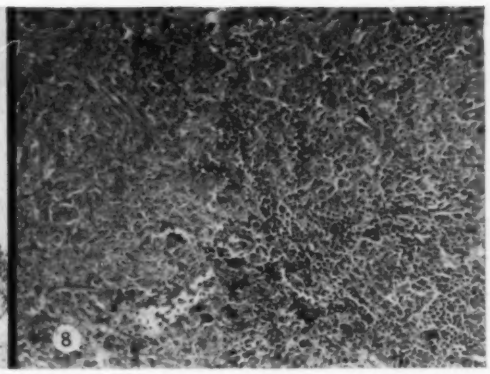


Fig. 8.—Brucellosis 21 days. Spleen. Epithelioid proliferation in pulp invading follicles. Giemsa stain; reduced about ⅕ from mag. × 180.

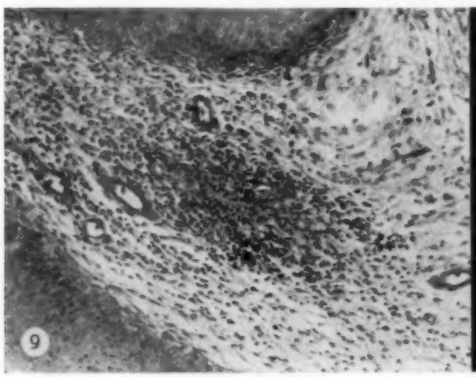


Fig. 9.—Brucellosis 21 days. Renal pelvis. Granuloma in connective tissue. Giemsa stain; reduced about ⅕ from mag. × 180.

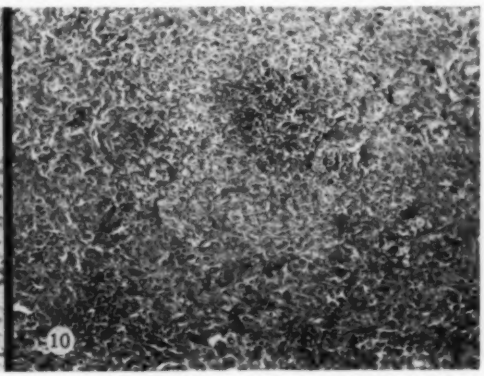
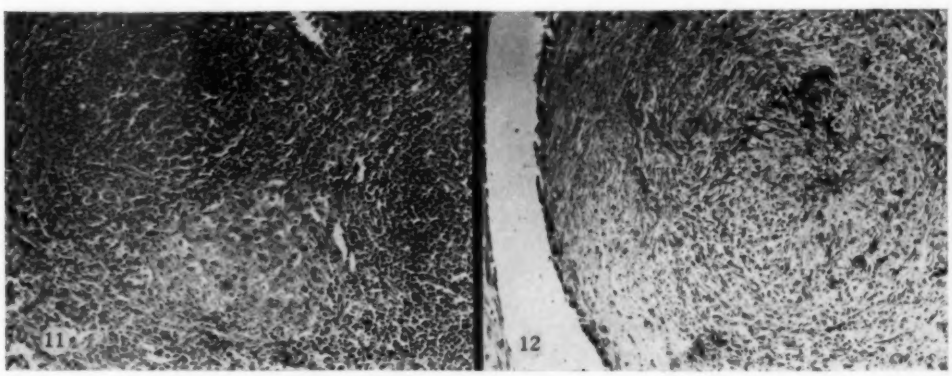


Fig. 10.—Brucellosis 17 days. Tracheal lymph node. Granuloma with epithelioid cells and necrosis. Giemsa stain; reduced about ⅕ from mag. × 180.

Fig. 11.—Brucellosis 266 days. Spleen. Fresh granuloma of epithelioid cells. Slight fibrosis in pulp. Giemsa stain; reduced about ⅕ from mag. × 180.

Fig. 12.—Brucellosis 266 days. Renal pelvis. Fresh granuloma in scarred stroma. Erosion of mucosa. Giemsa stain; reduced about ⅕ from mag. × 180.



necrotic. Between 17 and 56 days granulomata were found in femoral bone marrow. After four months healing with only slight scarring was evident in tracheal and cervical lymph nodes, when granulomata and even abscesses abounded in other tissues. In occasional instances fresh granulomata appeared in otherwise completely healed spleens at eight months (Fig. 11) or in the renal pelvis (Fig. 12).

Histological Changes in Guinea Pigs Exposed to Aerosol Containing C. Burnetii

At four days the lungs were only mildly edematous (Table 2). By 10 days the exudate involving alveolar and bronchial spaces and walls consisted of polymorphonuclear leucocytes with a few large mononuclear cells and fibrin (Fig. 13). Elementary bodies were not seen. The only other noteworthy lesions were nodules of mononuclear cells about the ducts in the Harderian glands (Fig. 14) and small granulomata of epithelioid cells in tracheal lymph nodes with intense pericapsular infiltration by epithelioid and mononuclear cells (Fig. 15). The intense perilymphadenitis readily distinguished this lesion from that of brucellosis (compare Fig. 1). Focal interstitial infiltrations of mononuclear cells in the renal pelvis and cortex were noted 10 to 56 days after exposure. By two weeks Billroth cords of the spleen were thickened by masses of mononuclear cells with vesicular nuclei, pale eosinophilic cytoplasm, and distinct polygonal outlines (Fig. 16). The sharply outlined cell wall also distinguished these cells from those in the brucellosis granuloma (compare Fig. 4). The perilymphadenitis about the tracheal lymph nodes extended widely through the mediastinal fat (Fig. 17). At 17 days granulomata appeared in bone marrow (Fig. 18), persisting only until 21 days, and in spleen (Fig. 19) and consisted of large mononuclear cells with distinct polygonal borders. From 4 to 14 days the bone marrow had increased numbers of neutrophiles. At three weeks granulomata were found around coronary arteries (Fig. 20) and in the mediastinal fat around tracheal lymph nodes (Fig. 21). The granulomata were characterized by giant cells, large polygonal mononuclear cells, and fat spaces within multinucleated giant cells, resembling those described in guinea pigs injected intradermally with psittacosis virus.[5] Granu-

Table 2.—*Gross and Microscopic Lesions in Guinea Pigs Infected with C. Burnetii* *

Days	Lymph Nodes				Spleen	Lung	Liver	Renal Pelvis	Bone Marrow	Lacrimal Gland
	Submental	Cervical	Inguinal	Tracheal						
4	..	..	..	..	..	1	..	..	>	..
4	..	..	..	..	..	1	..	..	>	..
10	M	M	..	M	1	1	..	I	>	I
10	M	M	..	M	..	2	..	..	>	..
14	2M	2M	..	1M	2G	±	FN	I	..	..
14	1M	2M	..	2M	2G	2	FN	..	..	I
17	1M	1M	2M	3M	1G	1	..	..	..	..
17	1M	3RE	2M	2M	4G	±	..	..	G	I
21	..	1RE	1RE	3RE	2G	±	G	G	G	..
21	1RE	1RE	1	1	3G	..	FN	..	G	..
27	4RE	2RE	G	GP	..	±	G	G	..	..
27	2GP	2R	GP	GP	..	1	..	I	..	..
41	..	..	..	..	..	..	..	I	..	..
41	..	..	G	..	..	..	..	I	..	..
56	1	..	1	..	..	..	..	I	..	..
56	1	1	..	..	1	..	..	I	..	..
118	..	..	..	..	..	..	..	..	..	..
118	..	..	..	..	..	..	..	..	..	I

* For explanation of symbols, see footnote to Table 1.

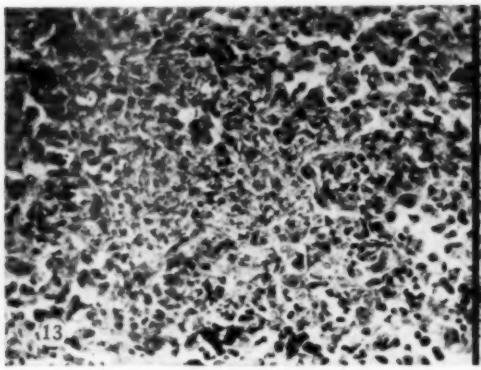


Fig. 13.—Q fever 10 days. Lung exudate of polymorphonuclear and mononuclear leucocytes and fibrin in alveoli. Giemsa stain; reduced about ⅕ from mag. × 385.

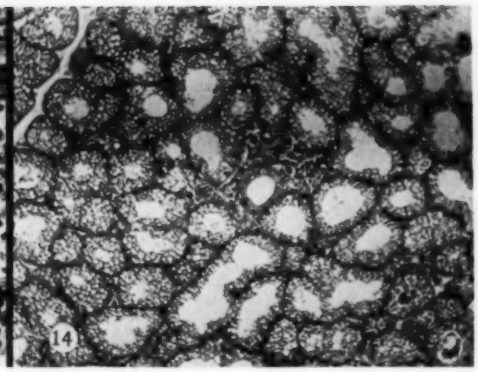


Fig. 14.—Q fever 10 days. Harderian gland. Nodule of polygonal-shaped mononuclear cells around duct. Giemsa stain; reduced about ⅕ from mag. × 180.

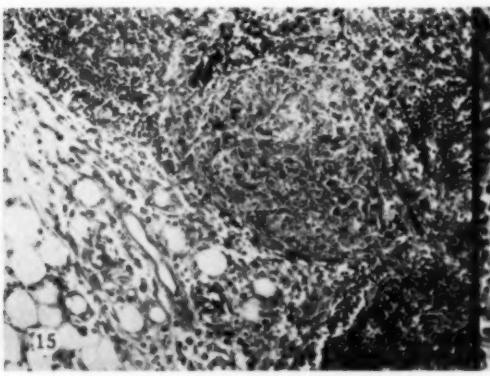


Fig. 15.—Q fever 10 days. Tracheal lymph nodes. Granuloma of epithelioid cells and pericapsular infiltration by mononuclear and epithelioid cells. Giemsa stain; reduced about ⅕ from mag. × 180.

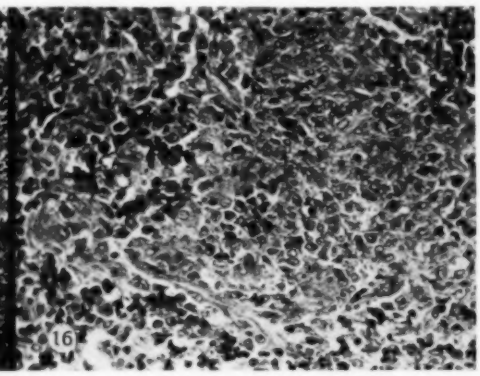
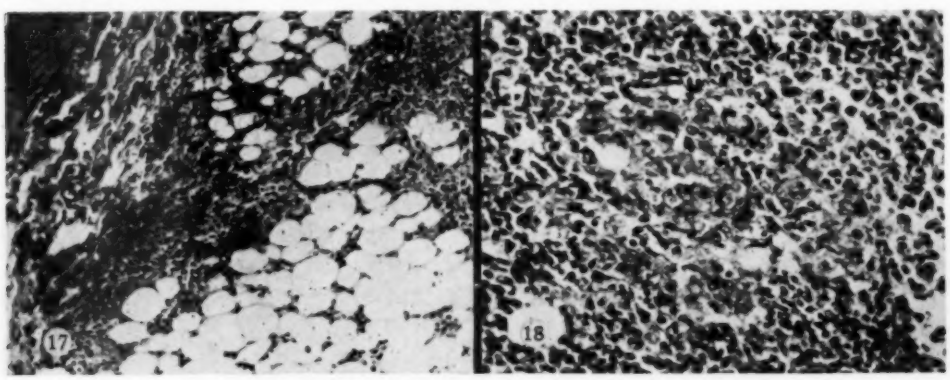


Fig. 16.—Q fever 14 days. Spleen. Epithelioid cells have distinct borders, are polygonal-shaped, and widen pulp cords. Giemsa stain; reduced about ⅕ from mag. × 385.

Fig. 17.—Q fever 14 days. Mediastinum. Exudate of mononuclear and lymphoid cells and fibrin. Collagen swelling. Giemsa stain; reduced about ⅕ from mag. × 180.

Fig. 18.—Q fever 17 days. Bone marrow. Granuloma of epithelioid cells and multinucleated giant cells. Giemsa stain; reduced about ⅕ from mag. × 385.



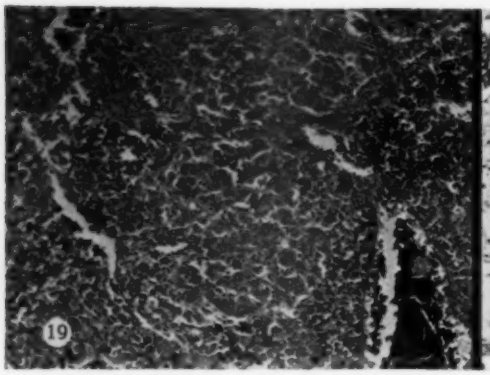


Fig. 19.—Q fever 17 days. Spleen. Granuloma of polygonal distinctly outlined epithelioid cells in pulp. Giemsa stain; reduced about ⅙ from mag. × 180.

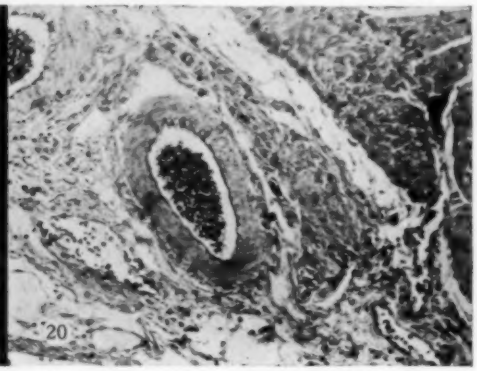


Fig. 20.—Q fever 21 days. Heart. Granuloma of epithelioid and giant cells around coronary artery. Giemsa stain; reduced about ⅙ from mag. × 180.

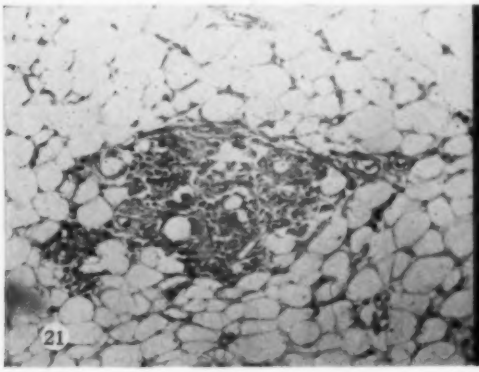


Fig. 21.—Q fever 21 days. Mediastinum. Granuloma of epithelioid and giant cells. Giemsa stain; reduced about ⅙ from mag. × 180.

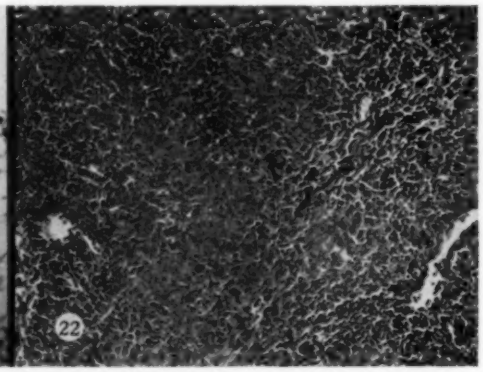
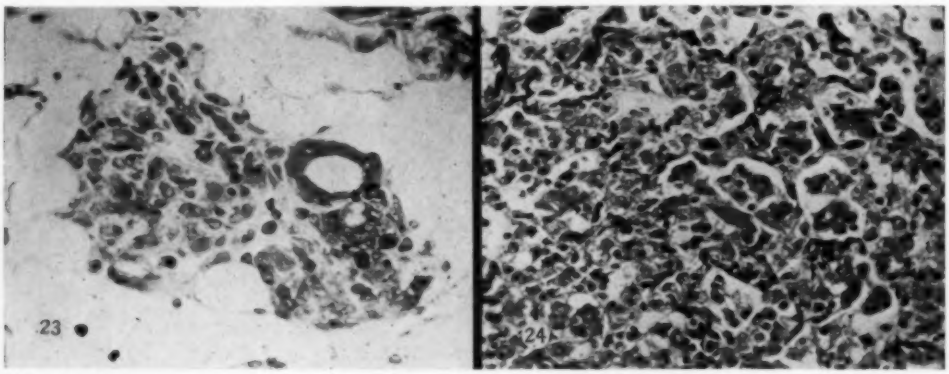


Fig. 22.—Q fever 21 days. Cervical lymph nodes. Granuloma of epithelioid cells with distinct cell borders. Giemsa stain; reduced about ⅙ from mag. × 180.

Fig. 23.—Q fever 27 days. Mediastinum. Granuloma of epithelioid and giant cells containing fat and a waxy pigment. Giemsa stain; reduced about ⅙ from mag. × 385.

Fig. 24.—Brucellosis and Q fever 14 days. Interstitial pneumonia. Exudate of fibrin and mononuclear cells. Giemsa stain; reduced about ⅙ from mag. × 385.



lomata also occurred in the pulp of cervical lymph nodes (Fig. 22). At four weeks the mediastinal granulomata contained a blue-green pigment in Giemsa stain, which was brown in the Kinyoun modification of the Ziehl-Neelsen stain (Fig. 23), differing from the ceroid of the psittacosis granuloma.[6] Lesions associated with Q fever were not found after four weeks except for infiltrates of lymphoid and mononuclear cells in the renal pelvis.

Histological Changes in Mixed Brucellosis and Q Fever in Guinea Pigs

At four days there was alveolar and bronchial infiltration of polymorphonuclear leucocytes but there were no elementary bodies (Table 3). Mediastinal lymphatic channels were dilated with polymorphonuclear and mononuclear leucocytes, but lymph nodes were unaltered. By two weeks the alveolar exudate was mainly mononuclear cells and fibrin (Fig. 24). Changes in tracheal lymph nodes, spleen (Fig. 25), and mediastinal fat (Fig. 26) were identical with those in Q fever (Fig. 19). Granulomata in the bone marrow appeared earlier than in Q fever or brucellosis, persisting from 14 to 41 days, but only at 14 to 17 days did they resemble those of Q fever. Interstitial tissue of the renal pelvis showed mainly lymphoid and mononuclear cell infiltration and rarely granulomata. At 17 days pulmonary lesions were regressing, and granulomata appeared in the respiratory lymph nodes with definite characteristics of brucellosis, namely, masses of pale-staining mononuclear cells with indistinct outlines, polymorphonuclear cell infiltration, and caseous necrosis. Intense perilymphadenitis like that of Q fever (Fig. 27) was also noted. After 21 days the tissue changes were indistinguishable from those of brucellosis, with necrotic granulomata in lymph nodes, spleen, liver, renal pelvis, epididymis, and endocardium. At four weeks there was more scarring and necrosis than was usually seen in brucellosis at this time, although later the distribution and progress of lesions was the same for the eight months of observation.

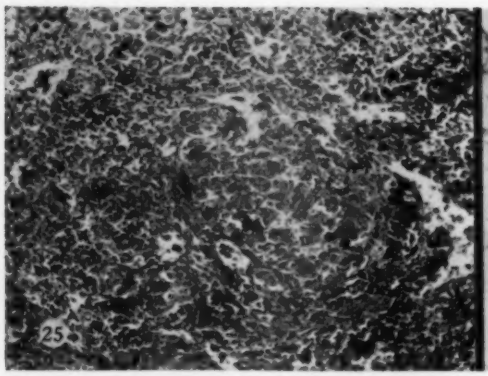
Table 3.—*Gross and Microscopic Lesions in Mixed Brucellosis and C. Burnetii in Guinea Pigs* *

Days	Lymph Nodes				Spleen	Lung	Liver	Renal Pelvis	Bone Marrow	Lacrimal Gland
	Submental	Cervical	Inguinal	Tracheal						
4	..	..	..	..	..	1	..	..	..	..
4	..	..	M	M	..	..	..	..	>	..
10	M	M	M	1M	I	2	FN	..	>	..
10	..	2M	..	M	I	±	FN	I	>	..
14	3RE	2RE	1RE	2RE	3RE	4	G	..	G	..
14	1RE	3RE	1RE	3RE	3RE	1	FN	I	G	..
17	1RE	2RE	1RE	4RE	4RE	±	G	RE	G	I
17	2RE	3RE	1RE	2G	4RE	±	G	I	FN	I
21	2RE	5RE	3RE	5G	4G	±	G	G	G	..
21	3G	6G	4RE	5G	4G	±	G	I	G	..
27	4G	4G	3G	3G	3G	..	F	I	G	..
27	4RE	4FRE	2RE	3G	6G	1	FN	I	G	..
41	4G	6GF	3G	4G	6G	1	G	I	G	G
41	3RE,F	5RE,F	4RE	4GF	4G	1	G	G	..	G
56	2G	2GA	2G	3GA	5GA	..	FN	I	..	I
56	2G	2GA	3G	4GF	4G	2	..	I	..	I
118	..	GF	F	F	2F	..	..	..	..	..
118	..	F	2	..	1	..	..	..	..	..

* For explanation of symbols, see footnote to Table 1.

Comparison of the Extent of Lesions in Guinea Pigs Exposed to Aerosols Containing B. Suis or C. Burnetii Separately or Combined

Table 4 summarizes the extent of lesions in lymph nodes, lungs, and spleen. The increases in lymph node size were recorded as



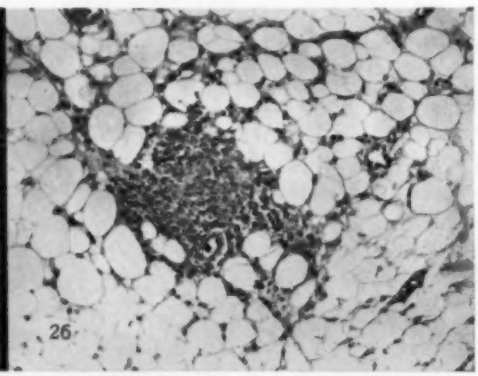


Fig. 25.—Brucellosis and Q fever 14 days. Spleen. Granuloma of distinctly outlined epithelioid cells similar to that in Q fever alone, in Figure 16. Giemsa stain; reduced about ⅓ from mag. × 180.

Fig. 26.—Brucellosis and Q fever 14 days. Mediastinum. Granuloma of epithelioid cells as in Q fever. Giemsa stain; reduced about ⅓ from mag. × 180.

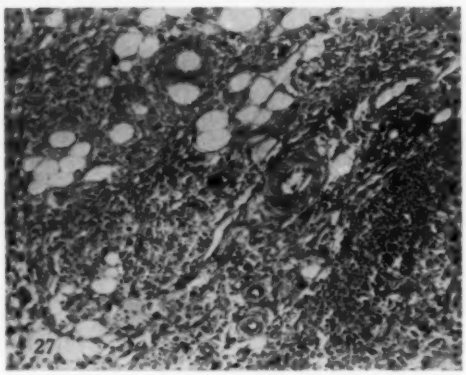


Fig. 27.—Brucellosis and Q fever 17 days. Tracheal lymph node. Infiltration of pericapsular fat and connective tissue with epithelioid, mononuclear, and lymphoid cells as in Q fever, Figure 15. Giemsa stain; reduced about ⅓ from mag. × 180.

TABLE 4.—*Gross Pathological Changes in Guinea Pigs Exposed to Aerosols of Combined or Separate B. Suis and C. Burnetii*

Day of Infection	Total Lymph Node Changes			Lung			Spleen		
	B*	BC*	C*	B	BC	C	B	BC	C
4	0	0	0	0	1	1	0	0	0
4	0	0	0	0	0	1	0	0	0
10	0	1	0	0	2	1	0	1	1
10	0	2	0	0	±	2	0	1	0
14	6	8	5	1	4	±	0	3	2
14	8	8	5	1	1	1	0	3	3
17	9	8	7	2	±	1	3	4	1
17	12	8	8	3	±	±	3	4	4
21	12	15	5	2	±	±	4	4	2
21	22	18	4	1	±	0	4	4	3
27	22	14	6	±	0	±	3	3	0
27	16	13	4	±	1	1	4	6	0
41	18	17	0	1	1	0	8	6	0
41	17	15	0	1	1	0	6	4	0
56	11	9	2	0	0	0	4	5	0
56	14	11	2	±	2	0	4	4	1
118	5	2	0	0	0	0	1	1	0
118	11	1	0	1	0	0	4	2	0

* B, infection with B. suis; BC, infection with B. suis and C. burnetii; C, infection with C. burnetii.

the arithmetic sum of the relative increased sizes of the submental, cervical, inguinal, and bronchial nodes. The figures for lung and spleen changes are as recorded in the previous Tables. Lymph node enlargement appeared earlier in the combined than in the separate infections. After three weeks lymph nodes were smaller in the combined infection than in brucellosis. Splenic enlargement in the various conditions paralleled that of the lymph nodes. The pulmonary lesions differed in brucellosis and Q fever in their time of onset and general histological character. The combined infection in the lungs went through the characteristics of both diseases. Neither the extent nor the type of pulmonary reaction was modified by the combined aerosol exposure. The anatomical data demonstrated that although the pulmonary lesions were unaffected by combining aerosols containing B. suis and C. burnetii the extent of generalized lesions due to B. suis was diminished.

COMMENT

The lesions of brucellosis in guinea pigs were far more extensive than those of Q fever. However, combined infection with B. suis and C. burnetii produced less extensive lesions and milder illness [1] than infection with B. suis alone. The combined infection had a lower and shorter period of fever, weight loss, and leucopenia.

Leucopenia in Q fever occurred between 13 and 15 days [1] and was not associated with bone marrow changes, although between 4 and 10 days an increase in neutrophiles was apparent in the marrow. However, with the onset of leucocytosis between 17 and 21 days, bone marrow granulomata of Q fever were present. Thereafter, leucocytosis was not associated with detectable lesions in the marrow. Although bone marrow granulomata were seen in brucellosis between 21 and 56 days, leucopenia was observed between 15 and 43 days.[1] Bone marrow granulomata in the combined infection were present between 14 and 41 days, yet leucopenia was present only between 13 and 25 days. Thus, the physiological responses only partially paralleled the structural changes in the bone marrow. The same conclusion with regard to structural and physiological changes is indicated in the generalized reaction to the disease.

The morphological data disclosed that the milder illness of the combined infection was a dual infection with less tissue damage. Since abundant tissue free of lesions in diseased organs was available for reaction sites, there was no evidence that competition for substrates or infection sites accounted for the milder disease.

Previous infection with B. suis decreased the mortality of lethal doses of C. burnetii or the recoverability of the Rickettsia when it was injected intraperitoneally with B. suis [1]; however, observations per se are insufficient to establish interference between the infectious agents, since both diseases resulted from exposure to both agents. On the other hand, the data show that both infections mutually diminish the host tissue reactions.

The diminished host response to mixed infections by C. burnetii and B. suis contrasts sharply with the aggravated illness in certain combined viral and bacterial infections [4] or with the interference phenomenon of mixed viral infections.[2] Resistance to C. burnetii after 11 days of infection with B. suis indicated that enhanced resistance to reinfection by one agent is not conclusive evidence of previous infection by the same or a closely related agent, as reported by McEwen [3] and others * for brucellae. Similar effects on resistance from other infections have been observed in unpublished studies. The mechanism of resistance in the dual infection described in the present studies is not explained by current theories.

The present data indicate that variation in infection rates in different experiments with the same agent may be due to the presence of unrelated infections in the tested animals. Depending upon the character of the primary infection of the host, infections may be increased, decreased, or even prevented. These possibilities demonstrate the necessity for employing animal colonies free of extraneous infection and for thorough examination of the host response in titrating infectivity of an agent.

CONCLUSIONS

Guinea pigs infected by exposure to aerosols containing Brucella suis or Coxiella burnetii, separately or combined, showed milder disease, and less extensive and more rapid healing of lesions in the combined infection than in the separate infection with B. suis.

In general, the symptoms and signs of the disease paralleled the extent of lesions only to a limited degree. There was considerable time lag between the occurrence of leucocytosis or leucopenia and recognizable bone marrow alterations such as increased myelopoiesis, focal necrosis, or specific granulomata. Furthermore, apparently recovered animals showed extensive lesions of brucellosis.

* References 5 and 6.

Resistance produced by one infectious agent to infection by another cautions against interpreting protection against reinfection as a specific reaction and indicates the necessity for care in selecting animals for studies with certain infectious agents.

Resistance to the manifestations of disease resulting from combined infection was not accounted for by the "interference phenomenon" or by any defined mechanism.

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Granulomatous Growth Induced in Mice by Absidia Corymbifera

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Fungi belonging to the family Mucoraceae are widespread in nature, where they frequently appear as saprophytes on decaying vegetation, on fruit in storage or in the market, and on dung of many species of animals. At least one species, Absidia corymbifera (Mucor corymbifer), is pathogenic for man and animals. This fungus was named Mucor corymbifer by Cohn,* and its pathogenicity was studied first by Lichtheim.[1] Vuillemin segregated this and related species pathogenic for man and animals in a new genus, which he named Lichtheimia.[2] The criteria upon which this generic name was based have been found trivial and unreliable by modern mycologists, and most of the specific names applied to isolates from human and animal lesions appear to be synonymous. However, A. corymbifera differs from a true Mucor and is properly placed in the genus Absidia Tieghem, 1876.† Despite this revised nomenclature, the disease caused by this fungus is generally called mucormycosis.

A. corymbifera is well known in human pathology for the acute fatal infection which it produces in the lungs ‡ and the central nervous system.§ Ocular || and dermal [15] infections have also been reported. Some infections attributed to Mucor cannot be accepted as mucormycosis in the absence of cultures and on the basis of morphologic evidence reported. The dichotomous branching, septation, or other characteristics of the fungus suggest Aspergillus rather than Absidia in several cases.¶ In other cases the structures observed must be identified as another fungus or an artifact.# Mucormycosis is also well known in animals,* although the evidence for an etiologic relationship is not complete in all the cases cited. Spontaneous or experimental infection of mice with A. corymbifera has not been reported. A peculiar tumor-like growth induced by this fungus in a mouse and found accidentally during a cancer research experiment stimulated the present study.

MATERIALS AND METHODS

In the previously mentioned cancer study C3H mice were fed by stomach tube 0.5% methylcholanthrene dissolved in olive oil. A 12-week-old male mouse which received a single feeding of 0.5 cc. of the oil developed a subcutaneous tumor six months later in the left pectoral region. The tumor had grossly all the characteristics of a mouse mammary tumor. It was firm and was about 3 cm. in diameter. Other lesions were not found at autopsy.

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National Cancer Institute and National Microbiological Institute, National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare. Present address of Dr. Symeonidis: Cancer Clinic, George Washington University Medical School.

* Cohn, in Lichtheim.[1]

† References 3 and 4, and in Hesseltine, C. W.: Personal communication to the authors.

‡ References 5 through 8.

§ References 9 through 12.

|| References 13 and 14.

¶ References 16 through 18.

References 19 and 20.

* References 21 through 30.

Because a mammary tumor was not expected in a male mouse following this exposure to methylcholanthrene, the animal was killed by ether, and small fragments of the grayish-white tumor tissue were transplanted subcutaneously in the pectoral region in 12 male 8-week-old mice of the same strain. The remainder of the tumor was fixed in Zenker's fluid and embedded in paraffin. In seven of the mice inoculated with the tumor transplants, tumors developed at the site of inoculation and, within 20 to 25 weeks, presented the same features as the original growth. Microscopic examination of the latter revealed a cellular granulomatous tissue containing a fungus. On the basis of this observation, cultures from the growth which developed in the inoculated mice were made. Pure cultures of A. corymbifera were obtained.

Experimental reproduction of the tumor-like growth by inoculation of mice with spores of this fungus was then attempted. These experiments were further extended by investigating the possibility of transformation of the proliferating granulomatous tissue into true neoplasia. This was attempted by feeding experimentally infected mice with a carcinogenic substance, 2-acetylaminofluorene (AAF). This carcinogen fed to animals produces tumors in various organs.[31] The carcinogenic action of this compound is frequently manifested in proliferating tissue.

A. corymbifera was isolated in culture on modified Sabouraud's agar slants (1% neopeptone, 2% glucose, and 1.5% agar), and this medium was used for subcultures from which spore suspensions were made. For the experimental inoculations spores were washed off the surface of the agar slant by pipetting isotonic saline (0.85% NaCl) over the slant, rubbing the surface with a stiff bacteriologic loop, and filtering the resulting suspension of spores through sterile cotton packed loosely into a small funnel. These operations were conducted under a bacteriologic hood. The filtered suspension was checked microscopically for freedom from mycelial fragments, and the spores were counted in a Levy counting chamber. Appropriate dilutions were then made, and in some experiments (as indicated) an adjuvant such as agar or mucin was added.

EXPERIMENTS

Four series of experiments were carried out. All mice used in these experiments were C3Hb mice (without the milk factor) 8 to 12 weeks old. They were fed Purina Chow pellets, with the exception of those fed the carcinogenic diet, and all animals had free access to food and drinking water. Tissue implantation to subcutaneous tissue in the pectoral area was done by trocar, according to the usual methods for tumor transplantation.

Series I.—Twelve male mice received subcutaneously tissue fragments from the original growth. In five subsequent passages five male mice were inoculated in each passage.

Series II.—Three groups of 48 male mice each were injected subcutaneously in the dorsoscapular area with spores of A. corymbifera. Spores were suspended for Group A in agar, for group B in mucin, and for Group C in saline. Within each group, eight subgroups of six mice each were injected with 50, 100, 250, 500, 10,000, 250,000, or 500,000 spores suspended in the respective medium (Table 1). All mice were killed 24 to 30 weeks after inoculation, because it was observed that when granulomas developed they appeared within a period of 12 to 15 weeks.

TABLE 1.—*Development of Granulomas in Male Mice Inoculated Subcutaneously with Absidia Corymbifera Spores Suspended in Three Media*

Group	Suspension Medium	Number of Spores Injected Subcutaneously							
		50	100	250	500	10,000	100,000	250,000	500,000
A	Agar	4/6 *	4/6	5/6	6/6	6/6	6/6	6/6	6/6
B	Mucin	0/6	1/6	2/6	4/6	6/6	6/6	6/6	6/6
C	Saline	0/6	1/6	3/6	5/6	6/6	6/6	6/6	6/6

* The number to the left of the line indicates the number of mice which developed granulomas. The number to the right of the line indicates the number of mice inoculated.

Series III.—Two groups of 12 male mice each were injected intraperitoneally with 1 cc. per mouse of a saline suspension of spores. One group received 1000 and the other 10,000 spores. The animals were killed 30 weeks after the inoculation.

Series IV.—The effect of combined exposure to the fungus and a carcinogen was tested in five groups of 38 mice each. Males and females were approximately equally represented. In Groups A, B, and C approximately half the animals from each group were injected subcutaneously with 100 spores, and the remainder with 1000 spores of A. corymbifera suspended in saline. Mice in Groups D and E were injected with 1 cc. of saline as controls. Groups A and D were fed Purina Chow. Groups B, C, and E were fed Derwood powder food containing 0.03% 2-acetylaminofluorene (AAF). In Group B the animals were inoculated with the spores at the time they were started on the carcino-

genic diet, while the mice of Group C were started on the AAF diet 12 weeks prior to inoculation with the fungus spores (Table 2). All mice in Series IV were 8 to 10 weeks old at the beginning of the experiment. In Group C the mice were started on the carcinogenic diet at this age and were inoculated with the fungus at 20 to 22 weeks of age.

Mice receiving the carcinogenic diet were killed when they became moribund, and mice from the other groups were killed to match these. When the experiment was terminated, the average age of the mice at time of death was 70 weeks. When mice were killed, specimens for histologic study were fixed in Zenker's acetic fluid, embedded in paraffin, and stained with hematoxylin and eosin, Masson's trichrome, and periodic acid-Schiff stains. Reticulum was demonstrated by the method of Wilder.

peritoneal cavity in the form of a polypoid tumor-like mass. These probably arose from spores deposited accidentally in the subcutaneous tissues during intraperitoneal inoculation. The remaining 24 mice were free of any lesions in the peritoneal cavity or elsewhere, but cultures taken from the peritoneal cavity of these animals were positive for A. corymbifera 24 weeks after inoculation.

Series IV.—In all Groups A, B, and C inoculated subcutaneously with fungus spores, a few mice injected with 100 spores in saline solution developed subcutaneous nodules. In the groups injected with 1000 spores the percentage of positive results was high

TABLE 2.—*Production of Granulomas in Mice Injected Subcutaneously with Absidia Corymbifera and Fed with 2-Acetylaminofluorene (AAF)*

	Absidia Corymbifera Spores Injected							
	Group A, Spores Only		Group B, Spores Plus AAF Feeding *§		Group C, Spores Plus AAF Feeding †§			
Number of Spores	Male	Female	Male	Female	Male	Female	Saline Only	Saline Plus AAF Feeding §
100	1/10 ‡	2/9	1/11	0/9	0/10	0/10	0/38	38/38 tumors
1,000	9/10	7/10	7/10	5/8	4/8	5/10	0/38	38/38 tumors

* 2-Acetylaminofluorene feeding started at the time of the fungus inoculation.
† 2-Acetylaminofluorene feeding started 12 weeks prior to fungus inoculation.
‡ The number above the line indicates the number of mice which developed granulomas. The number below the line indicates the number inoculated or treated.
§ All mice fed AAF developed tumors.

RESULTS

Series I.—In 7 of 12 mice receiving tissue fragments of the original lesion and in 15 of 25 inoculated with tissue fragments in five subsequent serial passages, subcutaneous masses developed which became palpable 12 weeks after implantation. In seven of these mice the tumor-like lesion reached a diameter of 3 to 4 cm. 24 weeks after implantation.

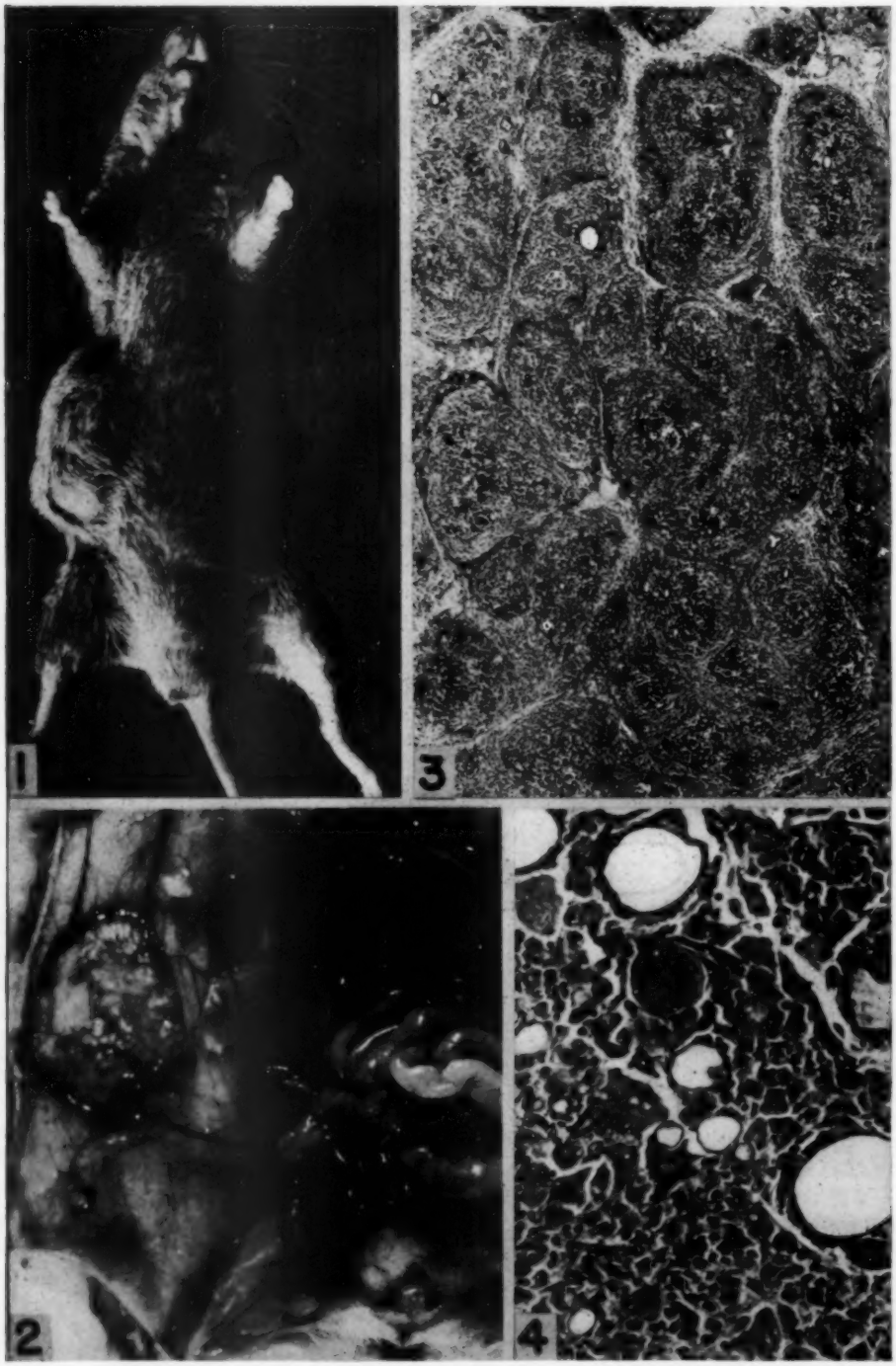
Series II.—Subcutaneous inoculation of 10,000 or more spores of A. corymbifera produced grossly visible or palpable subcutaneous nodules in all injected mice regardless of the suspension medium. Results with small numbers of spores varied with the medium (Table 1).

Series III.—One mouse injected intraperitoneally with 1000 spores and three with 10,000 spores developed subcutaneous growths. In two mice the nodule protruded into the

(Table 2). In mice fed the carcinogenic substance, especially in those started on the AAF diet before the fungus inoculation, the percentage of growths was lower (Table 2). In 9.6% of the animals with growths there was a proliferation of the lesion in the peritoneal cavity and metastases in the regional lymph nodes. In 16.3% the subcutaneous lesions regressed and the decrease in diameter of the granuloma was associated with death of the fungus, as indicated by the appearance of fungus cells in sections. All mice fed a diet containing AAF developed tumors, first in the liver and urinary bladder and subsequently in other organs. Neoplastic transformation of mycotic granulomas was not observed in any animal fed AAF.

GROSS PATHOLOGY

Most of the lesions were roughly spherical, and some reached a diameter of 3 cm.



Figures 1 to 4

(See legends on opposite page)

They were located subcutaneously and had the familiar appearance of a mammary tumor in mice (Fig. 1). The growth consisted of a compact, grayish-white, opaque tissue with scattered yellowish spots (Fig. 2), and it was easily removable, surrounded in the majority of the animals only by a thin capsule. Only exceptionally were there adhesions of the skin or the underlying tissues, or both. Ulceration developed in 12% of the granulomas. In 9.6% of the nodules the lesion infiltrated the retroperitoneal tissues and polypoid tissue masses protruded into the peritoneal cavity. In these animals the kidneys, the liver, and, in one mouse, the lungs were surrounded by granulomatous tissue masses. The parenchyma of the organs was not infiltrated, however. The serosal layer of the organ appeared to be a kind of protective barrier. In mice in which the fungus growth regressed, a flat mass of brownish-yellow friable tissue remained in its place.

MICROSCOPIC PATHOLOGY

The growth when fully developed consisted of a conglomeration of granulomas, which were quite clearly outlined by a network of thin fibrous tissue strips (Fig. 3). The granulomas were cellular and consisted mainly of histiocytes of varying forms and sizes and of a large number of multinucleated giant cells (Figs. 4, 5, and 6). The latter were mostly adjacent to or included cells of the fungus, which were sharply outlined in sections stained with the periodic acid-Schiff stain (Fig. 5). Lymphocytes and a few plasma cells were present in the periphery of the granulomas. In places disintegrating hyphae and accumulations of leucocytes formed microscopic abscesses. In later stages involution of the granulomas could be observed, starting with disintegration and disappearance of the fungi. The granulomatous tissues became dense, and the cellular elements were principally uniform, fusiform fibroblasts. In this stage the granuloma could be mistaken for a neoplastic lesion, for instance, a fibroscarcoma, although the granulomatous nature of the lesion was still easily recognizable (Fig. 7). In a few animals there was dissemination to the lymph nodes, where the fungus produced characteristic granulomas, quite similar to tubercles, with large, poorly stained "epithelioid" cells and large multinucleated giant cells (Fig. 6).

The fungus in tissue has the morphologic characteristics usually associated with Absidia. The hyphae are large, coenocytic with few septa, and polymorphic, and they stain better with hematoxylin than hyphae of most pathogenic fungi. In these tumor-like lesions there is an unusual development of swollen, vesicular fungus cells containing little stainable cytoplasm and reaching a diameter of 50μ. It has not been determined whether this is a characteristic peculiar to the strain of fungus or whether it is related to the host and conditions of inoculation.

In culture A. corymbifera grows rapidly and forms on the agar surface a short, mouse-gray, turf-like colony in which the aerial portion is composed of branching hyphae and erect sporangiophores. The hyphae are multinucleate and have very infrequent septa. The fungus has the type of corymbiform branching, sporangium, columella, and spore characteristic of A. corymbifera (Fig. 8). Dr. C. W. Hesseltine confirmed this specific identification.

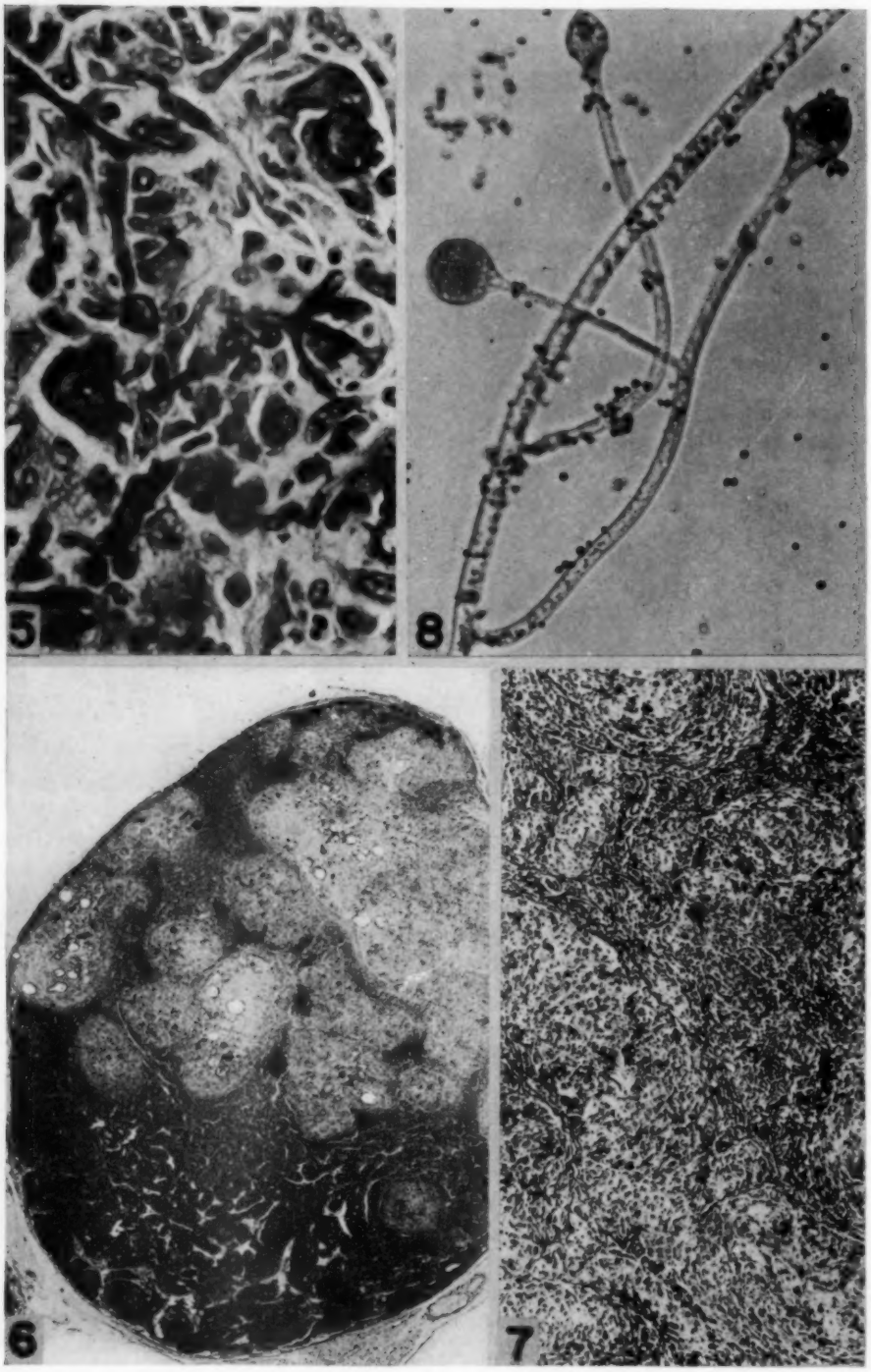
EXPLANATION OF FIGURES 1 TO 4

Fig. 1.—Mouse inoculated subcutaneously with 100,000 spores of A. corymbifera suspended in saline. Granulomatous growth 2.5×1.5×1.8 cm. at the site of inoculation after 25 weeks.

Fig. 2.—Same mouse as in Figure 1 at autopsy. Granulomatous growth at left with fine granulated surface.

Fig. 3.—Cellular granulomas of varying form and size, outlined by thin strands of connective tissue. The fungi are hardly visible at this low magnification in the form of darkly stained spots in the granulomas. Periodic acid-Schiff stain; × 31.

Fig. 4.—Pleomorphic cellular granuloma consisting of histiocytes of varying form and size, giant cells, lymphocytes, and leucocytes with sections of distended fungus hyphae. Hematoxylin and eosin; × 200.



Figures 5 to 8

(See legends on opposite page)

COMMENT

A. corymbifera, as revealed in present experiments, has in the mouse a low degree of infectiousness. It proliferates locally in the subcutaneous tissue and induces formation of an excessive amount of granulomatous tissue, but there is no apparent general toxic effect in the animal. The granulomatous lesion could be produced by subcutaneous inoculation of fungus spores. Even with 50 spores it was exceptionally possible to induce a lesion. A minimum of 1000 spores was necessary in order to produce nodules regularly. The microscopic features were those of a foreign-body granuloma. Grossly this granuloma had a striking resemblance to a true neoplasm; namely, it showed constant proliferative growth leading to a large tumor-like structure which infiltrated the surrounding tissues and even the peritoneal cavity in the form of polypoid tissue masses. "Metastases" in the regional lymph nodes occurred not infrequently, and the growth was transplantable to other mice, to which the fungus was transmitted with the tissue fragments. The peak of the proliferative period of the lesion was reached 16 to 25 weeks after inoculation, and the nodule reached a diameter of 3 cm. Subsequently, the lesion either remained stationary or regressed and disappeared. The only forms of spread observed were by slow infiltration of the surrounding tissues, invagination of the peritoneal cavity, and dissemination to the regional lymph nodes with production of tubercle-like granulomas. Widespread dissemination by the lymph or the blood stream to distant organs was not observed. Granulomatous lesions were produced only when the fungus was inoculated subcutaneously. When introduced intraperitoneally, the fungus remained latent. It could be recovered in cultures 24 weeks after intraperitoneal inoculation, but it did not produce lesions. This would indicate that the injected spores in the peritoneal cavity do not find a proper substrate for their further development and that they do not release toxic and lytic substances which would damage the coelomic epithelium. The source and manner of infection in the mouse with the original lesion are unknown. Approximately six months after the discovery of the original fungus growth, the same type of lesion developed "spontaneously" in a C3H mouse fed pure olive oil by stomach tube (control animals). It is known that this fungus is a common saprophyte. Possibly, therefore, a spore was accidentally introduced into the subcutaneous tissue of the mouse through a superficial skin injury made during the manipulation of the animals for the feeding experiment.

SUMMARY

A tumor-like subcutaneous granuloma induced in the mouse by Absidia corymbifera is described. Grossly this growth gave the impression of a true neoplasm. It proliferated slowly and constantly and extended into the surrounding tissues, as well as into the peritoneal cavity. It was transmissible to other mice by subcutaneous inoculation of tissue fragments. With the exception of the regional lymph nodes, the lesion did not extend to other organs. Histopathologically it was very cellular and consisted of histiocytes and numerous multinucleated giant cells. The lesion could be produced exceptionally with 50 spores of the fungus injected sub-

EXPLANATION OF FIGURES 5 TO 8

Fig. 5.—Numerous hyphae in form of irregular tubes with swellings and constrictions and with lateral branches. Adjacent to the hyphae are multinucleated giant cells and histiocytes. Periodic acid-Schiff stain; × 565.

Fig. 6.—Section of lymph node with numerous tubercle-like granulomas in which fungus cells appear like vacuolated areas. Periodic acid-Schiff stain; × 20.

Fig. 7.—Cellular granuloma in early stage of regression. Fungus hyphae are no more demonstrable. Large bundles of fibroblasts are surrounding macrophages loaded with lipid substance. Periodic acid-Schiff stain; × 100.

Fig. 8.—Hyphae, sporangia, columellae, and sporangiospores of A. corymbifera from a culture.

cutaneously into mice and regularly with 100 spores. Direct intraperitoneal injection of spores did not produce the lesion.

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Lateroposition of the Atrial Appendages

A Case of Levoposition of the Appendages

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While reviewing the autopsied cases of transposition of the great vessels indexed at Henry Ford Hospital, an unusual case was seen which seemed worthy of a separate report. In this heart the atria * were normally situated, but their appendages * were displaced to the left, both lying on the left side of the transposed great vessels (Fig. 1). Such a striking departure from the normal is of considerable anatomical interest, although of little clinical significance except insofar as it occurs in association with other anomalies. A generally accepted name has not been given this anomaly. In this report a case is presented together with a study of the anomaly and a suggested nomenclature.

Fig. 1.—Drawing of the heart showing its anterior aspect. Both atrial appendages are seen to the left of the large pulmonary artery. The right appendage is shown held back to reveal the left coronary artery at its origin. Note the small right ventricle, hypoplastic ascending aorta and arch, preductal coarctation, and distal patent ductus continuing into the descending aorta.

REPORT OF CASE

N. F., an underdeveloped 7-month-old Negro girl, was noted to be cyanotic only on crying. The family history was interesting in that the child's paternal great-grandmother and grandmother and father were known to have some sort of congenital heart defect. On examination, there was a loud systolic murmur audible all over the precordium. Femoral pulses were present. Fluoroscopy showed increased pulmonary vascular markings and enlargement of the heart—chiefly left ventricular enlargement. The EKG showed left ventricular preponderance. While the child was being studied, atelectasis of the left lung developed. At bronchoscopy the lower part of the trachea was seen to be compressed and the left main stem bronchus was completely collapsed at its origin. The child died a few days later.

At autopsy the heart was enlarged, weighing 75 gm. The apex and most of the anterior surface of the heart were formed by the left ventricle, the right ventricular component being very small.

The right atrium was large, measuring 20 mm. in all diameters. It received the superior and inferior venae cavae normally. The sulcus terminalis lay very far forward, so that the lateral wall of the atrium was

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* In the Birmingham Revision of the Basle "Nomina Anatomica" the heart chamber first receiving the venous blood is termed atrium, and its ear-like process, auricle. In older clinical writings the terms auricle and appendage are used respectively for these structures. In current clinical literature the terms atrium and atrial appendage have largely superseded the others and will therefore be used in this report.

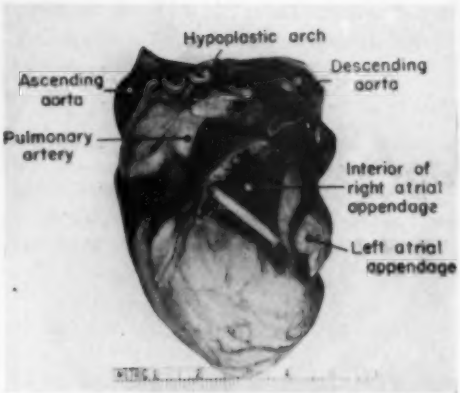


Fig. 2.—Left anterior oblique view of the heart showing the interior of the right atrial appendage.

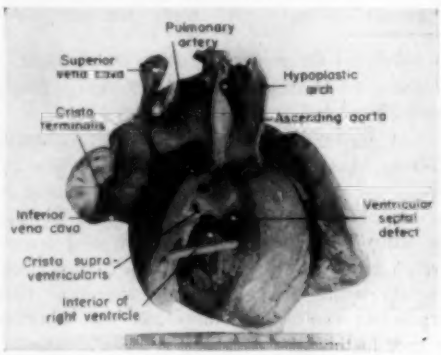
formed almost entirely of that part derived from the sinus venosus. There was no appendage on the right side. In its place was a small triangular space in which the main pulmonary trunk and the right pulmonary artery could be seen lying posteriorly between the transposed aorta and the superior vena cava. The interior of the right atrium was almost entirely smooth-walled, except for a few musculi pectinati in front of the anteriorly placed crista terminalis. There were two openings in the atrial septum. The foramen ovale measured 8×8 mm. Below it was a fenestrated foramen primum measuring 6×6 mm. Above and anterior to both foramina was the entrance to a tunnel-like extension of the right atrium, measuring 8×10 mm. in width. This passed to the left behind the great vessels forming the posterior wall of the transverse sinus and ending in the displaced "right" appendage. This appendage was large, measuring 15 mm. in width and 20 mm. in length. It was densely trabeculated and filled with old blood clots. The appendage lay just to the left of the transposed pulmonary artery at its origin from the left ventricle (Fig. 2). A large coronary sinus entered the right atrium in the usual position, its ostium measuring 4×3 mm. In the floor of the atrium was a small tricuspid valve, 9 mm. in diameter, leading into a very small right ventricle.

The cavity of the right ventricle measured 20 mm. long by 10 mm. wide. It communicated with the left ventricle through a high ventricular septal defect, measuring 7×3 mm. Some of the chordae tendineae of the septal leaflet of the tricuspid valve were attached to the posterior and inferior margins of the ventricular septal defect. The average thickness of the right ventricular muscle was 5 mm. A prominent crista supraventricularis lay between the right ventricular inflow and outflow tracts (Fig. 3).

The aorta originated from the right ventricle. It measured 10 mm. in diameter up to the origin of the innominate artery, where a sudden decrease in diameter occurred affecting the entire arch. At the origin of the left subclavian artery the diameter of the aorta was 2 mm. This width continued to the point of entry of the ductus arteriosus, which was patent and measured 2 mm. in diameter. Beyond the ductus the aorta widened to a diameter of 9 mm. (Fig. 1). The aortic valve presented the pattern seen typically in complete transposition of the great vessels, the acoronary cusp being anteriorly placed.

The posterior descending branch of the right coronary artery lay opposite the posterior edge of the ventricular septum. The anterior descending branch of the left coronary artery lay about 5 mm. to the left of the anterior edge of the septum.

Fig. 3.—Interior of the right ventricle showing the ventricular septal defect and the crista supraventricularis. Note the anterior position of the crista terminalis, the smooth atrial wall behind, and the musculi pectinati in front of it.

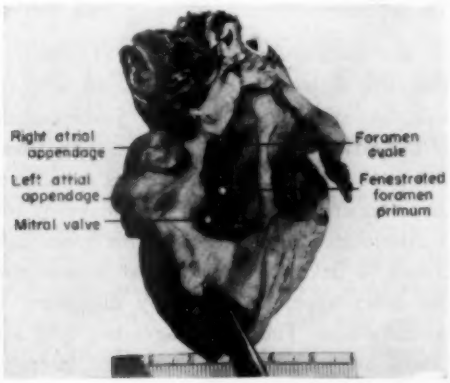


The left atrium received the pulmonary veins normally; it was smaller than the right, measuring some 16 or 17 mm. in all diameters. The left appendage, smaller than the displaced right appendage, lay just below and to the left of it and measured 12 mm. in width and 15 mm. in length. The mitral valve was normal, measuring 15×13 mm. across. The left atrium communicated with the right by the previously described foramina (Fig. 4).

The left ventricular cavity measured 36 mm. long by 20 mm. wide. The average thickness of the muscle was 7 mm. The left ventricle communicated with the right by the previously described ventricular septal defect (Fig. 5).

The pulmonary artery arose from the left ventricle. It measured 20 mm. in diameter, being twice as wide as the ascending aorta. The valve was tricuspid. The artery passed upward and to the right behind the aorta. At the point where it divided into the right and left branches, the artery ended in a dome-like dilatation which compressed the bifurcation of the trachea and the left main bronchus. At its origin the left pulmonary artery was connected to the aorta by the patent ductus arteriosus. Other findings were not remarkable except for complete atelectasis of the left lung.

Fig. 4.—Left aspect of the heart showing the interior of the left atrium. Note the large patent foramen ovale and the small fenestrated foramen primum. Both atrial appendages are visible; the left is partially opened to show its continuity with the left atrium.



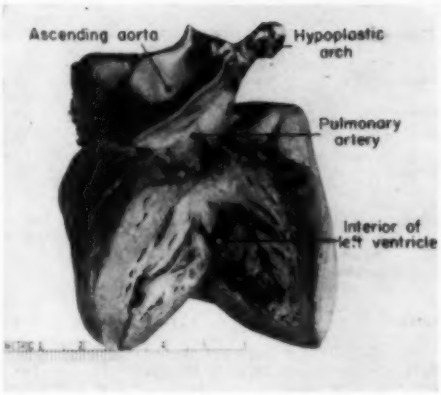


Fig. 5.—Interior of the left ventricle. Compare with the right ventricle (Fig. 3). Note the larger cavity and thicker muscle of the left ventricle.

COMMENT

In this case we have in a minimally cyanotic child showing left ventricular preponderance on the EKG the association of complete transposition of the great vessels; atrial septal defects; hypoplasia of the right ventricle, ascending aorta, and arch; preductal coarctation, and patent ductus arteriosus. This syndrome forms a clinically significant entity, to be described fully elsewhere.[1] The malposition of the atrial appendages in this case was associated with complete transposition of the great vessels, although it is more commonly found in association with partial transposition of the great vessels.†

The literature on this subject has been recently reviewed by Dixon.[3] He described 2 cases of his own, 1 in which the appendages were on the right, the other in which they were on the left, and reviewed 10 other cases with the appendages on the left.‡ In addition to these cases and the one described in the present report, four others have been reported with the appendages on the left,§ and one other with the appendages on the right. The latter case was briefly described by Abbott[2] in 1936. It is included in this report, although the exact position of the atrial appendages is not clearly described.

† References 2 and 3.

‡ References 4 through 12.

§ References 13 through 16.

		Age	Sex	Cyanosis	EKG	Transposition of Great Vessels	Great Veins	A. S. D.
A. Levoposition								
1. Birmingham	1898	20 yr.	F	Yes	.....	Partial	Persistent left S. V. C.	Large
2. Wenner	1900	10½ yr.	F	Yes	.....	Partial	Persistent left S. V. C.	Large
3. Dünner	1914	10 wk.	..	Yes on crying	.....	Partial	Three pulmonary veins	Small & small foramen primum
4. Huebschmann	1921	5 mo.	M	Yes	.....	Partial	Normal	Large
5. Kettler	1932	1 yr.	M	Yes	.....	Partial	One pulmonary vein left S. V. C., no right S. V. C.	Large
6. Ngai	1935	1½ mo.	F	Yes	.....	Partial	Normal	Yes
7. Bredt	1935	5 yr.	F	....	.....	Partial	Normal	Yes
8. Bredt	1935	*	..	....	.....	.....	.....	.....
9. Harris & Farber	1939	1 yr.	M	Yes	.....	Partial	Normal	Yes
10. Taussig	1947	26 yr.	F	Yes	Left axis deviation	Partial	No I. V. C., hepatic vein entered left atrium	Large
11. Miskall & Fraser	1948	11 mo.	F	Yes	.....	Complete	Normal	Large
12. Rogers, Cordes, & Edwards	1950	12 yr.	M	Yes	Left axis deviation	Complete	Normal	Large and fenestrated foramina prima
13. Dixon	1954	7½ yr.	F	Yes	Left axis deviation	Partial	One pulmonary vein	Large
14. Edwards, et al.	1954	7½ yr.	F	Yes	Right ventricular hypertrophy	Complete	Persistent left S. V. C.	Large
15. Polanco & Powell	1955	3 wk.	M	Questionable on crying	Right ventricular preponderance	Complete	Normal	Large
16. Smyth	1955	7 mo.	F	Yes on crying	Left ventricular preponderance	Complete	Normal	Yes, & small fenestrated foramen primum
B. Dextroposition								
1. Abbott	1936	20 yr.	M	Yes	Complete heart block, right ventricular preponderance	Complete	Additional right S. V. C. entering left atrium	No
2. Dixon	1954	3 wk.	F	Yes	.....	Partial	All enter left atrium	Large & small foramen primum

Explanation of symbols: ..., not described; *, case identical with No. 7 but not described; A. S. D., atrial septal defect (includes patent foramen ovale); V. S. D., ventricular septal defect; P. D. A., patent ductus arteriosus; S. V. C., superior vena cava; I. V. C., inferior vena cava; L. V., left ventricle; R. V., right ventricle.

The essential features of these cases are listed in the Table.

There is no generally accepted name for this anomaly. Dünner [6] (1914) in describing his case used the phrase, "transposition of the two auricular appendages." Ngai [13] (1935) referred to "sinistroposition of the right auricle." Bredt [9] (1935) in describing his first case mentioned "side by side position of the appendages." Abbott [2] (1936) used the terms "dextroposition" and "sinistroposition of the auricles." Dixon [3] (1954), noting that the displaced appendages lie side by side, has suggested the name "juxtaposition of the atrial appendages." The essential feature of this anomaly, however, is the lateral displacement of one atrial appendage by the malpositioned great vessels.[5] This appendage comes to lie between the great vessels and the other appendage, thereby displacing it laterally also. The appendages not only are placed in juxtaposition, they are laterally

of the Atrial Appendages

V. S. D.	P. D. A.	Aortic Hypoplasia	Pulmonary Arterial Hypoplasia	Aortic Arch	A-V Valves	Ventricles	Persistent Bulbus	Remarks
Large	No	No	Valvular stenosis	Right	Normal	L. V. small	Yes	Dextrocardia situs solitus
Large	Small	No	Yes, bicuspid valve	Left	Transposed	R. V. small	Yes	Dextrocardia situs solitus
Large	Large	Yes	No	Left	Normal	R. V. small	Yes	Preductal coaretation of aorta
Small	Small	No	Yes, bicuspid valve	Right	Tricuspid atresia	R. V. small	Yes	Dextrocardia situs solitus
Large	No	No	Yes, bicuspid valve	Left	Tricuspid atresia mitral valve, five leaflets	R. V. small	Yes	.....
Large	Small	No	No	Right	Normal	R. V. small	Yes	.....
Large	No	No	Yes, atresia	Left	Normal	R V. small	Yes	.....
.....	.....	....	.....	.....	.....	.....	....	.....
Large	Small	No	Yes	Left	Normal	R. V. small	Yes	.....
Large	No	No	Yes	Left	Common valve	R. V. small	Yes	Dextrocardia situs inversus
No	No	No	No	.....	Mitral valve small	.....	No	.....
Yes	No	No	Yes, infundibular stenosis bicuspid valve	Left	Tricuspid atresia	R. V. small	No	.....
One small, one large	No	No	Yes, infundibular stenosis	Left	Normal	R. V. small	Yes	.....
Yes	.....	....	No	.....	.....	.....	No	.....
Small	Large	Yes	No	Left	Tricuspid atresia	R. V. small	Yes	Dextrocardia situs solitus, hypoplasia of aortic arch, preductal coaretation
Yes	Yes	Yes	No	Left	Tricuspid valve small	R. V. small	No	Hypoplasia of aortic arch, preductal coaretation
Complete	Yes	Yes	No	Left	Double mitral valve	R. V. small	Yes	Dextroposition of atria, position of left appendage not described
Yes	Large	No	No	Left	Normal	R. V. small	No	Preductal coaretation of aorta

displaced. It would seem better, therefore, to call this anomaly "lateroposition of the atrial appendages." The two varieties would then be "dextroposition" and "levoposition of the atrial appendages."

Reference to the Table will show that the anomaly of lateroposition of the atrial appendages is associated with the clinical feature of cyanosis and the anatomical features of partial or complete transposition of the great vessels, septal defects, and a small right ventricle. In the cases associated with partial transposition of the great vessels, there is persistence of the bulbus which may form a so-called third ventricle.[8] Less commonly there may be diminished pulmonary blood flow (10 of 18 cases), dextrocardia in situs solitus (4 of 18 cases), preductal coarctation of the aorta (4 of 18 cases), and various anomalies of the great veins and A-V valves.

The embryology of this anomaly has been discussed by Wenner,[5] Kettler,[8] Ngai,[13] and Dixon.[3] The mechanism of development of levoposition of the atrial appendages might be explained somewhat as follows. The normal development of the heart, during the

fourth and fifth weeks, is illustrated in Figure 6 by the sequence $A \rightarrow B1 \rightarrow C3$. During this time the truncus and bulbus move to a central position between the developing atrial appendages. With the division of the truncus late in the fifth and early in the sixth week,[17] this move permits the aorta, or, in complete transposition, the pulmonary artery, to reach the left ventricle. At the stage the bulbus disappears, being in part incorporated in the outflow tract of the right ventricle.[17] If for some reason, at about the

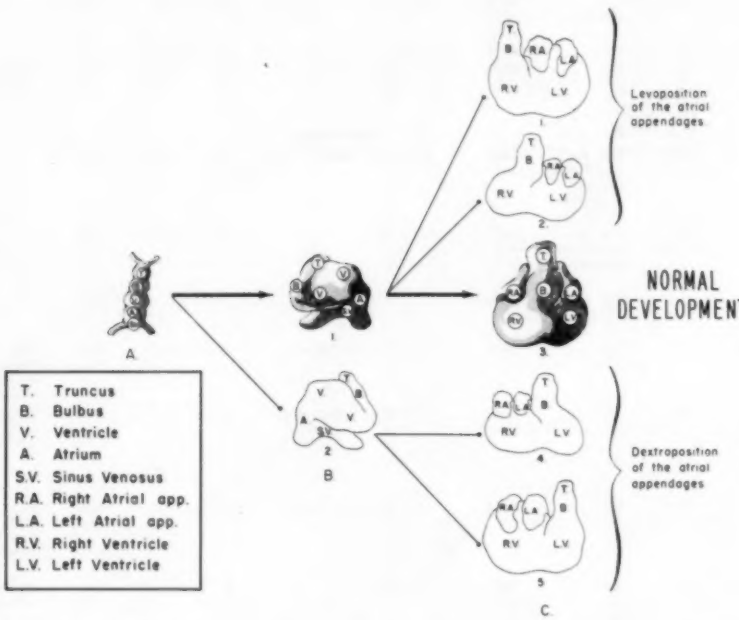


Fig. 6 (In part after Wenner [5] and Dixon [2]).—Possible sequence of events in the development of levoposition and dextroposition of the atrial appendages. *A*, 8-somite stage progressing to *B*, 16-somite stage during the fourth week; *C*, 5 mm. stage, fifth week, showing the various possibilities in development. For details see text.

16-somite stage, development ceases but growth continues, there will be persistence of the bulbus, which with the truncus fails to move toward the midline. Wenner [5] postulated that this sequence of events, illustrated in Figure 6 by the sequence $A \rightarrow B1 \rightarrow C1$, caused the atrial appendages to develop on the left side of the great vessels. In this situation the truncus does not reach the left ventricle, which then develops without an outflow tract other than a ventricular septal defect. Both great vessels then arise from the right ventricle, or one from a persistent bulbus which may be large enough to form a so-called third ventricle,[8] a condition described as partial transposition of the great vessels. Ngai [13] referred not only to persistence but also to "detorsion of the bulbus cordis," and a reference to the Table will show that lateroposition of the atrial appendages is always associated with transposition of the great vessels, partial or complete. In order to explain dextroposition of the atrial appendages, one must postulate a reversal of the bulboventricular loop before the 16-somite stage, as is shown in Figure 6 by the sequence $A \rightarrow B2 \rightarrow C5$. In complete or partial transposition of the great vessels one or both vessels arise from the distal ventricle, which is morphologically the right ventricle in a normally disposed bulboventricular loop. If both vessels arise from what appears to be the left ventricle, as occurred in Dixon's first case, this must have been the distal ven-

tricle, which came to be on the left because of a reversal of the bulboventricular loop. The fine trabeculation of the right ventricle and the coarse trabeculation of the left, described by Harris and Farber [10] as characteristic of bulboventricular inversion, were present in Dixon's case. Presumably there can be reversal of the bulboventricular loop without complete inversion, since the A-V valves in Dixon's case were normally placed. It is of interest that in this case there was also malrotation of the gut.

Although lateroposition of the atrial appendages is commonly associated with partial transposition of the great vessels—the appendages overlying the ventricle without an outflow tract—in almost one-third of the cases, including the one reported here, it was associated with complete transposition of the great vessels. In these cases, apparently, the bulbus had moved far enough centrally to allow the pulmonary artery to reach the left ventricle, but not far enough to allow the atrial appendages to develop normally. For these cases one might postulate an intermediate course of development such as is shown in Figure 6, $A \rightarrow B1 \rightarrow C2$ for levoposition and $A \rightarrow B2 \rightarrow C4$ for dextroposition of the atrial appendages.

There is a notable disparity in the incidence of levoposition and dextroposition of the atrial appendages. The disparity might be explained by the association of dextroposition of the atrial appendages with malrotation of the bulboventricular loop. This anomaly would presumably be much rarer than one involving arrested development of a normally disposed bulboventricular loop, this being the anomaly associated with levoposition of the atrial appendages.

These various schemes of development show how lateroposition of the atrial appendages can occur; they do not explain why it occurs so rarely. One would expect it to occur frequently in association with transposition of the great vessels, whereas, of course, in the great majority of cases of partial or complete transposition of the great vessels, the atrial appendages develop normally, in spite of the anomalous great vessels.

SUMMARY

A case is described in which multiple cardiac anomalies were found, the special feature being lateral displacement of the atrial appendages, both of which lay to the left of the great vessels. In the absence of a generally accepted name for this anomaly, lateroposition of the atrial appendages is suggested as a general term, the two varieties being levoposition and dextroposition of the atrial appendages. The literature on this anomaly, comprising reports on 15 cases of levoposition and 2 cases of dextroposition of the atrial appendages, is reviewed, and the essential features of all the cases are tabulated. The embryology of this anomaly is discussed and a scheme of development suggested for the known variants of this anomaly.

Dr. Conrad R. Lam and Dr. Robert F. Ziegler gave advice and suggestions in the preparation of this report.

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The Media of Small Muscular Pulmonary Arteries in Mitral Stenosis

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Hypertrophy of the muscle in the small pulmonary arteries of patients with mitral stenosis has been described frequently.* The conclusion that medial hypertrophy is present has been based largely on subjective methods. Parker and Weiss [1] described "some medial hypertrophy" in the small pulmonary arteries. Enticknap [2] diagnosed hypertrophy in the arteries and arterioles, usually of the media, by "simple microscopical inspection" in 11 of 32 lingular biopsy specimens taken at the time of mitral commissurotomy. Smith, Burchell, and Edwards [3] used a grading system, but apparently without actual measurement of vessels, and derived an "index of change" of "3.6" for the media of the small muscular pulmonary arteries in 10 patients who had died of rheumatic mitral stenosis. This "index of change" does not lend itself to precise translation, but, according to our interpretation based on their definitions, it would represent a 100% average increase in thickness of the media of the small pulmonary arteries.

In contrast, Goodale and Thomas [7] could not demonstrate medial hypertrophy in the pulmonary arteries in 30 cases of mitral stenosis from which biopsy tissues of lung had been obtained at mitral commissurotomy. They used a method based on the ratio, derived from measurements, of the thickness of the media to the external diameter of the vessel.

The ratio of the thickness of the media to the external diameter of the vessel is not an entirely satisfactory measure of the arterial musculature because this ratio varies with the state of contraction of the vessel. Unless the arteries measured are completely relaxed, their external diameters cannot fairly be used as a reference point for comparison of the thicknesses of their mediae. Because only rare vessels can be found in a completely relaxed state, as indicated by lack of wrinkling of the internal elastic lamina, we have attempted to devise another objective method for the measurement of arterial muscle.

Branching of the pulmonary arteries parallels the branching of the bronchial tree; so a practical basis for the selection of pulmonary arteries for comparison would be to choose only those closely associated with a clearly recognizable and constant subdivision of the bronchial tree. The respiratory bronchiole fulfills these requirements. To eliminate errors in measurement due to contraction, the actual cross-sectional area of the media can be calculated. It is the purpose of this study to apply these methods to a study of the pulmonary arterial media in mitral stenosis.

MATERIAL AND METHODS

Autopsies of 67 patients over 20 years of age with "moderate" or "severe" mitral stenosis were selected for study. A "normal" control group of 45 patients without cardiovascular disease was selected

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* References 1 through 6.

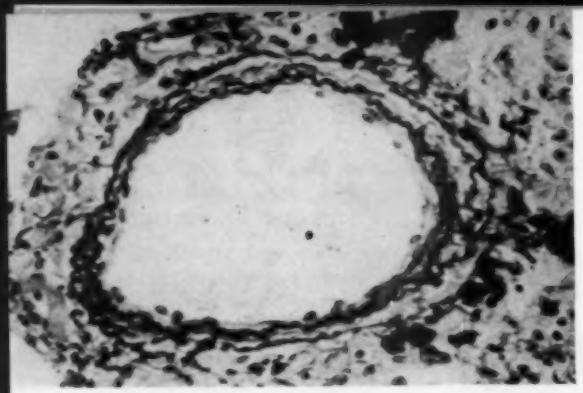


Fig. 1.—Class II small muscular artery near a distal respiratory bronchiole. There is little smooth muscle or elastic tissue in the bronchiolar wall. The media of the artery is sharply outlined by the internal and external elastic laminae. "Normal" control. Aldehyde fuchsin-Van Gieson-hematoxylin; × 283.

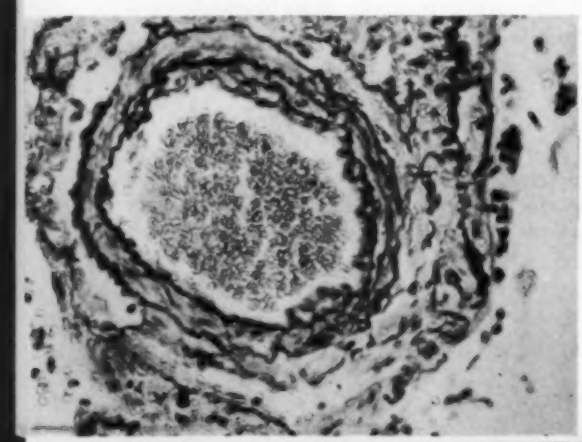
from recently performed autopsies on the basis of age, with an approximately equal number of cases in each decade from 20 to 100.

The average ages of the patients in the two groups were similar: 54 years (controls) and 55 years (mitral stenosis). The average cardiac weight in the patients with mitral stenosis was 532 gm. and in the control group 338 gm. The average thickness of the wall of the right ventricle of the heart was 6.7 mm. in the patients with mitral stenosis and 4.4 mm. in the controls.

The paraffin blocks of Zenker-formol-fixed tissue of lung from all patients were recut and stained with aldehyde fuchsin-Van Gieson-iron hematoxylin.[8] This procedure sharply stains the elastic laminae of the vessel wall and thereby clearly outlines the width of the media in the muscular pulmonary arteries.

Two classes of small muscular pulmonary arteries were chosen for measurement (Figs. 1, 2, and 3). The first (Class I) was comprised of those lying adjacent to the origin of a respiratory bronchiole. This portion of the respiratory bronchiole can be clearly identified by (1) a definite band of smooth muscle in the bronchiolar wall and (2) its tall columnar epithelial lining. The second (Class II) was composed of those lying adjacent to the peripheral respiratory bronchiole, recognized by (1) the paucity or absence of smooth muscle cells in the bronchiolar wall, which is composed almost entirely of fibrous and elastic tissue, and (2) the low columnar or cuboidal epithelial lining. The incomplete circumference of its wall characterizes the respiratory bronchiole at both levels, distinguishing it from the more proximal portions of the bronchial tree.

Fig. 2.—Class II small muscular artery near a distal respiratory bronchiole from a patient with mitral stenosis. Aldehyde fuchsin-Van Gieson-hematoxylin; × 283.



Only those vessels cut in a plane at approximately right angles to their walls were measured. Vessels were eliminated if their roughly circular outline was lost or if the elastica was "smeared," indicating an oblique cut.

Measurements were made microscopically, using a calibrated ocular scale. The average external diameter of the media of each artery was measured between the inner surfaces of the external elastica, and the area of a circle of this diameter was determined. Then the internal diameter of the artery was measured between the external surfaces of the internal elastica, and the area of this inner circle determined. The difference in the areas of these two circles represented the area of media in square micra.

RESULTS

In almost every case of mitral stenosis and in the "normal" controls, two vessels of each class were found that fulfilled the criteria for measurement.

Class I Small Muscular Arteries

Sixty-seven measurable Class I arteries, adjacent to muscular respiratory bronchioles, were found in the lung tissue sections of 37 "normal" controls. The average area of the mediae was 3361 square micra. One hundred ten Class I arteries were found in the tissue sections of 61 cases of mitral stenosis. The average area of their mediae was 3499 square micra. This difference in areas is not statistically significant (Table). (Discrepancies

Analysis of Results: Cross-Sectional Area of the Media of Small Pulmonary Arteries

	Class I Arteries		Class II Arteries	
	Control	Mitral	Control	Mitral
Mean areas in sq. micra	3,361	3,499	958	991
Number of cases.....	37	61	41	60
Standard deviation....	2,073	1,638	404	569
Standard error	341	210	79	73
95% confidence limits..	±692	±420	±160	±146
t	0.3		0.3	
p	>0.5		>0.5	
Detectable difference...	22%		22%	

between the total number of cases and the number of cases in which measurable vessels of a specific class were found are explained by the fact that vessels suitable for measuring could not be found in some cases.)

The average of the external diameters of these Class I arteries in the "normal" controls was 180μ; in the cases of mitral stenosis, 155μ. This difference in diameters is significant ($p < 0.01$).

The average of the internal diameters of these vessels in the controls was 169μ; in the cases of mitral stenosis, 141μ. This difference is also significant ($p < 0.02$).

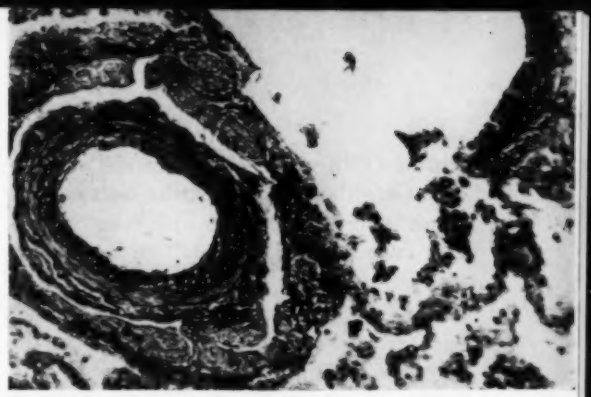


Fig. 3.—Low magnification of a Class I small muscular artery near a proximal respiratory bronchiole, illustrating the lack of continuity of the bronchiolar wall. The bronchiolar wall contains a heavy band of elastic tissue and smooth muscle. The arterial intima is thickened by fibrous tissue. Mitral stenosis. Aldehyde fuchsin-Van Gieson-hematoxylin; × 132.

Class II Small Muscular Arteries

Eighty measurable Class II arteries, adjacent to distal respiratory bronchioles, were found in the lung sections from 41 "normal" controls, and the average area of the mediae was 958 square micra. One hundred eighteen Class II arteries were found in 60 cases of mitral stenosis, and the average area of their mediae was 991 square micra. This difference in areas is not significant (Table).

The average of the external diameters of these Class II arteries in the "normal" controls was 104μ; in the cases of mitral stenosis, 90μ. This difference in diameters is significant ($p < 0.01$).

The average of the internal diameters of these vessels in the controls was 98μ; in the cases of mitral stenosis, 83μ. This difference is also significant ($p < 0.01$).

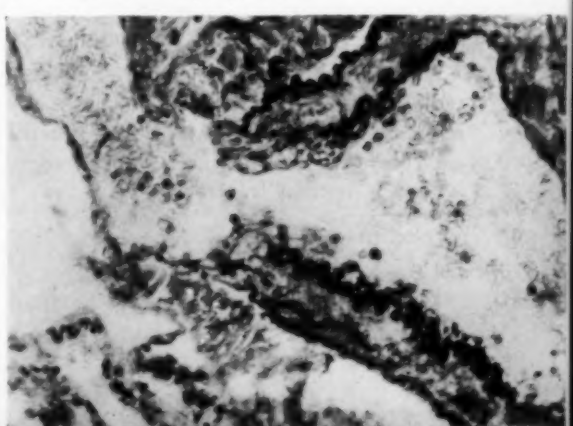
Arterioles.—The arterioles in every case were examined, and none possessed a media. Arteriolar walls at their point of origin from small muscular arteries (Fig. 4) were especially scrutinized, since only by the demonstration of this origin can a pulmonary arteriole be distinguished definitely from a venule.

Comment

Our method is based on three assumptions: 1. The branch of the pulmonary artery at the level of a respiratory bronchiole is constant enough to be selected for a comparative study. Analysis of our results supports this assumption, because the calculated difference in muscle area we could have theoretically detected between the "normal" controls and patients with mitral stenosis was 22% (Table). 2. The volume of muscle changes only slightly with contraction. This has been established with striated muscle [10] and probably applies to smooth muscle. 3. The length of a muscular artery does not change appreciably with contraction. This assumption can be made because the smooth muscle of the media is arranged circularly about the long axis of an artery. Accepting these three assumptions, the area of the media in a cross section of an artery is dependent upon the volume of muscle present, and would therefore remain constant regardless of the state of contraction of the vessel.

Using the method that has been presented for determining the amount of media in the small pulmonary arteries, no significant differences could be demonstrated between

Fig. 4.—Pulmonary arteriole arising from a small muscular artery. Contraction of the artery is evident from the wrinkling of the elastica. The muscular coat of the vessel ends abruptly at the origin of the arteriole. "Normal" control. Aldehyde fuchsin-Van Gieson-hematoxylin; × 366.



patients with mitral stenosis and "normal" controls without cardiovascular disease. This result is in disagreement with other studies that were based largely on subjective methods.

There is little doubt that average pulmonary arterial pressures were higher in the group of patients with mitral stenosis than in the group of "normal" controls. That we were unable to demonstrate muscular hypertrophy brings up the question of whether hypertrophy of arterial muscle (pulmonary or systemic) ever occurs as the result of an acquired condition.

In many cases of pulmonary hypertension resulting from congenital anomalies producing a shunt of blood from the left to the right side of the heart, there is definite persistence of fetal arteriolar media, but it has not been established that it represents a true "hypertrophy."† Normally the pulmonary arterioles lose their muscular coat early in infancy, and we have never seen arteriolar media that has developed as the result of an acquired lesion. However, occasionally a delicate and incomplete line of elastic tissue forms over a concentric fibrous intimal lesion, and this fibrous tissue might then easily be mistaken for muscle by the unwary.

The significant difference in diameters of small muscular arteries in these patients with mitral stenosis as compared with "normal" controls could explain the *apparent* increase in medial thickness of the pulmonary arteries that has been reported by other investigators in cases of mitral stenosis. If this difference in diameter actually represents a state of contraction prevailing during life, it may be in part responsible for the increased resistance to pulmonary blood flow often found in patients with mitral stenosis.

SUMMARY AND CONCLUSIONS

Small muscular pulmonary arteries were examined for evidence of medial hypertrophy in 67 autopsied patients with mitral stenosis and compared with the small muscular arteries in 45 "normal" controls.

Vessels were selected for measurement by virtue of their location near a respiratory bronchiole.

To avoid errors due to inconstant degrees of contraction of the arteries, the cross-sectional area of the media of each vessel was determined.

By this method a significant difference in the amount of arterial media could not be demonstrated in the two groups of cases.

Arteriolar media could not be identified in either group of cases.

These results fail to support the concept that arterial medial hypertrophy develops as the result of acquired arterial hypertension as suggested by previous investigators. Explanations for this apparent discrepancy are suggested.

The average diameter of comparable arteries was significantly less in the patients with mitral stenosis, suggesting a greater degree of arterial contraction in these cases.

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Intracranial Dissecting Aneurysms

An Analysis of Their Significance

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Although dissecting aneurysms occur most commonly in the aorta or its branches, they may also develop occasionally in other vessels. Very rarely they are found in the intracranial arteries. Since the number of cases in which the cerebral vessels are affected are so few, the addition of a new one and a review of the previously reported cases, together with an analysis of the manner of their development, seem timely.

A dissecting aneurysm is an arterial lesion in which, as a result of weakening, the vessel wall becomes split longitudinally into two layers by the leakage of blood beneath the weakened inner portion. The new channel is not lined by endothelium but by the tissues dissected. It is, therefore, a false aneurysm.

Not only are these intracranial lesions rarely encountered, but reports of them are difficult to find. Sinclair,[1] for instance, was able to find only four cases, while reporting one of his own. A casual perusal of the titles of the articles in the bibliography of this paper shows that in only three of the nine reports was there an adequate clue to the presence of an intracranial dissecting lesion.* A tabulation of these cases is presented in order to illustrate certain salient features or elements that were common to several, although their number is too small for definitive analysis.

Before proceeding further it seems pertinent to present an unusual case of an intracerebral dissecting aneurysm which occurred as an unfortunate complication following the

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* References 1 through 3.

Intracranial Dissecting Aneurysms

Case No.	Author	Sex	Age	Arteries	Cause	Thrombosis	Remarks
1	Scholefield (1924) [4]	M	47	Rt. vertebral and basilar	.....	—	Died 15 days after onset of cold
2	Hyland, Case 2 (1933) [5]	M	42	Basilar	.....	+	.....
3	Hassin (1937) [6]	M (N)	35	Basilar and its branches	Accidental electrocution	—	.....
4	deVeer & Browder (1942) [7]	M	42	Rt. middle cerebral	20 ft. fall to ground	+	Lived 57 hr.
5	Dratz & Woodhall (1947) [2]	F	21	Left internal carotid with adjacent anterior and middle cerebral	Hit by auto	+	Lived 3 days
6	Ramsey & Mosquera (1948) [3]	M	47	Rt. middle cerebral	Cystic medionecrosis and arteriosclerosis	—	Rupture of vessel
7	Poppen (1951) [8]	..	..	Angular branch of middle cerebral	.....	—	Seen on angiogram, surgically excised
8	Poppen, ibid.	..	..	Midcerebral	.....	—	Seen on angiogram; ligated internal carotid
9	Sinclair (1953) [1]	F	27	Rt. middle cerebral	.....	—	Migraine for several yr.
10	Bigelow, present case	F	46	Rt. middle cerebral	Excision of berry aneurysm	+	Lived 1 day postoperatively

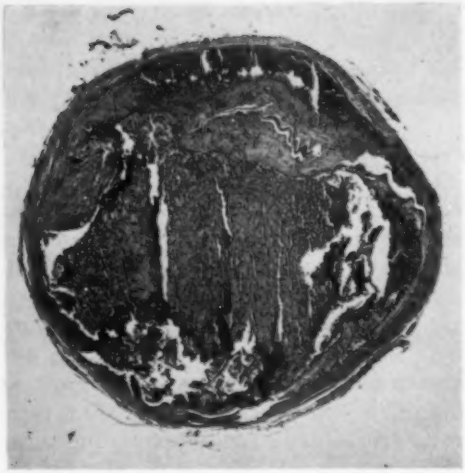


Fig. 1.—Cross section of right middle cerebral artery near operative site. The dissection involves nearly one-third of the circumference of the vessel at this point. Hematoxylin and eosin; reduced ⅓ from mag. × 45.

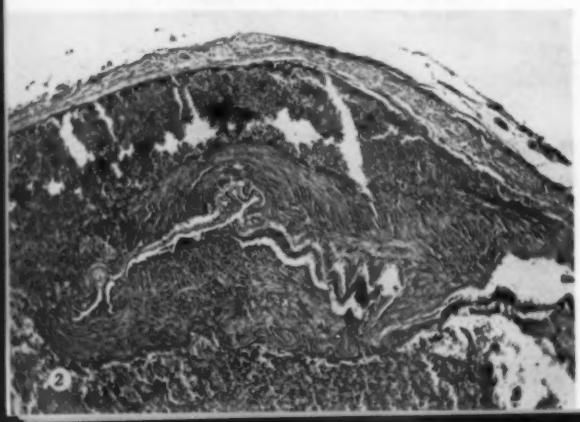
removal of a congenital, or berry-type aneurysm.

REPORT OF A CASE

The patient, a 46-year-old white woman, was admitted to the hospital because of a headache whose onset was sudden but which had been severe and persistent for 10 days. Clear xanthochromic spinal fluid was obtained on lumbar puncture. A right carotid angiogram revealed an abnormality of the middle cerebral artery, a mulberry-like shadow, suggestive of an aneurysm. A slight shift of the ventricular system to the left was observed by means of a pneumoencephalogram.

On the day after pneumoencephalography the patient's condition deteriorated rapidly and she became comatose. An immediate operation was performed. A right parietotemporal bone flap was turned down, and the right temporal lobe was resected. An aneurysm of berry-type, which originated from the middle cerebral artery at a point approximately 2 cm. from its origin from the internal carotid, was found. A small yellowish-red hematoma surrounded the aneurysm. The circulation of the middle cerebral artery was temporarily occluded for a period of one and one-half to two minutes, during which time the base of the aneurysmal sac was dissected free and removed, leaving a longitudinal opening about 0.6 cm. in length in the wall of the artery. This surgical defect in the wall of the vessel was closed by means of silver clips. Because the bleeding at the site of resection was uncontrollable, the clips were removed and the rent in the arterial wall was then closed by means of interrupted fine silk sutures (#000000). All other bleeding points were carefully obliterated. The dura was then partially closed, and the upper portion of the bone flap replaced. Postoperatively, the patient remained in coma, developed left hemiplegia, and died the following day.

Fig. 2.—Higher magnification of aneurysm to show extent of tear into media. Hematoxylin and eosin; reduced about ½ from mag. × 130.



Necropsy, performed three hours after death, showed early acute infarction of the right cerebral hemisphere supplied by the middle cerebral artery. No bleeding was observed at the suture sites of the resected aneurysm. The right middle cerebral artery, for a distance of 1 cm. distal to the suture, was dark purple in color due to the infiltration of the outer coats of the artery by blood. On section, the lumen of the vessel appeared to be occluded by a recent thrombus.

Serial sections of this artery disclosed the presence of a dissecting aneurysm at the operative site. Nearly one-third of the circumference of the vessel was involved, and the tear extended deep into the media (Figs. 1 and 2). There was no evidence of thrombus formation in this region. The dissection extended distally along the course of the vessel for a distance of 1.3 cm. By means of serial sections it was possible to show that the rent in the media became gradually more superficial and embraced a progressively smaller portion of the circumference of the vessel (Figs. 3 and 4) until it terminated at the intimal surface, at which point full continuity was restored. Curiously, at the end of the dissection farthest removed from the operative site early thrombus formation was present, and, just beyond the point where the intima was again intact, the lumen of the artery was entirely occluded by a recent antemortem thrombus (Fig. 5).

COMMENT

The paucity of reports on dissecting aneurysms of intracranial arteries permits of only tentative conclusions concerning their etiology and pathogenesis. The rarity of these lesions suggests, first, that there are certain characteristics of the intracranial arteries that make them relatively unlikely sites for this

disorder. Second, as seen in the tabulation of previously reported cases, it is unlikely that the conditions responsible for intracranial dissections are fundamentally similar to those which have been considered responsible for aortic dissections. The primary factors responsible for dissection of the aorta, according to Gore and co-workers,† are degenerative changes either of the elastic laminae or of the smooth muscle of the media, depending on whether the patient falls in a younger or older age group. Gore [10] also believes that a biochemical or metabolic disorder rather than a morphologic change is primary and that mechanical factors, such as hypertension or trauma, are of secondary importance.

Traumatic cerebral injuries precipitated intracranial artery dissection in 4 of the 10 tabulated cases, an incidence of 40%. On the other hand, when considering the great frequency with which head injuries occur, this is a conspicuously insignificant number. Dratz and Woodhall [2] suggested that a shearing or rotating type of injury might have been responsible for the dissection seen in their case. Whether or not some set of unusual conditions is necessary to produce a tearing effect in an artery is at best speculative. Probably any force, however applied, if it is to produce a dissecting aneurysm, must be of a rather specific degree of intensity: It must be forceful enough to cause a tearing of the vessel wall, yet not so violent as to produce complete disruption. For if there is complete transection of an artery, or merely a perforation, massive bleeding will effectively result in obscuring and obliterating the evidences of even a large vascular dissection. Indeed, it seems likely that numbers of tears of intracranial arteries have been overlooked because of the large hemorrhage produced by the rupture of the arterial wall. In this regard it is noteworthy that only Ramsey and Mosquera [3] have observed intracranial dissection, when there was rupture of the involved artery.

In the case reported here, the surgical trauma to the intracranial tissues was, relatively speaking, insignificant. Few causes other than some type of surgical manipulation would permit of such precise and effectively localized injury to the affected vessel so that dissection without complete rupture

† References 9 and 10.

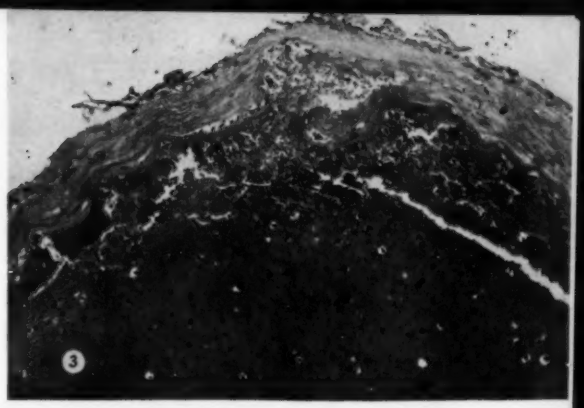


Fig. 3.—Aneurysm near point of termination. Here the size of the dissection is much smaller, but it still penetrates deeply into the muscular wall and there is disruption of the internal elastic lamina. Hematoxylin and eosin; reduced about ½ from mag. × 160.

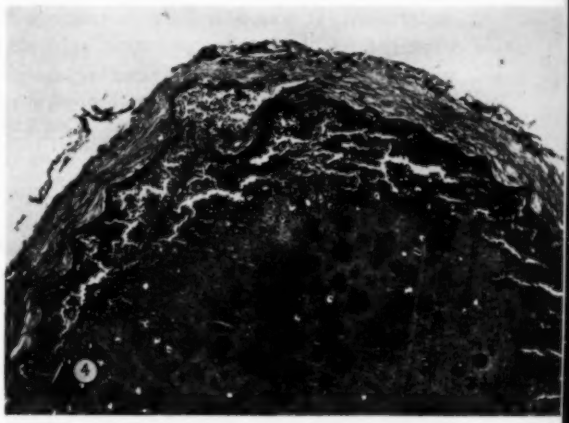
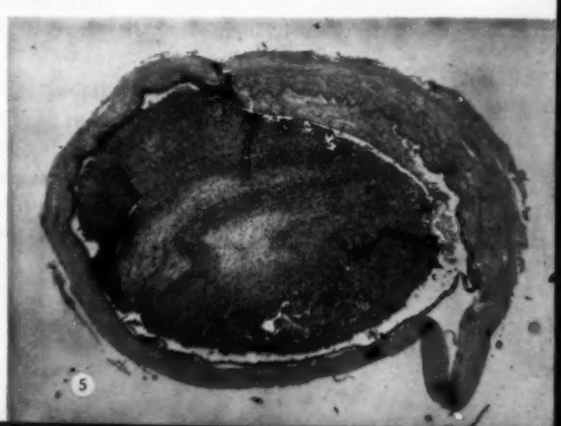


Fig. 4.—Same as Figure 3. The section is stained by Verhoeff elastic tissue stain to illustrate the break in continuity of the internal elastic lamina, reduced about ½ from mag. × 160.

Fig. 5.—Right middle cerebral artery just distal to dissecting aneurysm, showing occluding thrombus. Hematoxylin and eosin; reduced about ½ from mag. × 50.



would result. However, one other possible element in aneurysm formation should be kept in mind, namely, whether or not the berry aneurysm itself could have been a predisposing factor in the dissection. Such aneurysms occur in vessels with defective walls, and there may have been some significant relationship. On the other hand, the aneurysms might represent little more than an interesting coincidental lesion. In order to check the possible relationship of congenital aneurysms to the development of dissecting aneurysms, a review of the histological characteristics of the former was undertaken. It was found that a number of ruptured or "leaking" aneurysms exhibited some degree of dissection. Therefore, an investigation of dissection as it may pertain to the pathogenesis of rupture of berry aneurysms has been undertaken, the findings of which will be reported in another communication. A preliminary evaluation has indicated, however, that some degree of arterial dissection occurs in an appreciable percentage of bleeding intracranial aneurysms.

An important sequela to intracranial artery dissection has been the occurrence of thrombosis of the affected artery, as observed in 4 of the 10 tabulated cases. As is well known, inflammation, ulceration, or other conditions which compromise the integrity of the intima may lead to intravascular clotting. Hence, thrombotic occlusion of the lumen of the vessel represents a significant potential complication of dissection. Possibly a careful scrutiny of the literature on intracranial artery thrombosis would disclose other instances of dissection not recorded in this study. To be sure, thrombosis with subsequent extensive encephalomalacia would usually make the presence of dissection, even when present, seem of trivial import, to be mentioned merely in passing, if recorded at all.

In the case reported here, thrombus formation occurred near the termination of the dissection and complete thrombotic occlusion was at a point slightly distal to the lesion (Fig. 5). An occasional unfortunate complication following surgical treatment of an intracranial aneurysm is the development of encephalomalacia in the region supplied by the vessel from which the aneurysm arises. It is usually assumed that thrombosis or spasm is mainly responsible for the diminished circulation. Perhaps dissection followed by thrombosis or spasm is also a factor to be considered.

While cystic medionecrosis is often of major importance in the development of dissection of the aorta, only one instance of dissection presumably produced as a result of this peculiar degenerative change in a cerebral artery has been reported.[3] On the other hand, cystic medionecrosis occurs extremely infrequently in intracranial arteries.

Still another way in which dissecting aneurysms may develop has been proposed by Paterson [11] and by Wartman.[12] It has long been known that normally the intima and the inner region of the media have no vascular channels. However, the intima and media of arteries altered as a result of arteriosclerosis, syphilis, or other disorders often are abundantly vascularized. These newly formed capillaries are relatively fragile and may readily lead to spontaneous intramural hemorrhage which may produce rupture of the vessel or dissection of its wall. Possible important sequelae of such intramural bleeding include arteriospasm, thrombosis, or vascular hemorrhage. While both Paterson [11] and Wartman [12] specifically mention that intracranial artery dissection may be produced in the above-described manner, neither author seems to have encountered an instance of longitudinal splitting of fibers sufficiently striking to be worthy of special comment. In none of the 10 cases reviewed by me could the abnormal vascularization of the vessel wall, as described by Paterson and Wartman, be considered the cause of dissection.

Up to this point the phenomenon of dissection has been considered only as it applies to the large intracranial arteries at the base of the brain, including the circle of Willis. Yet, in the older literature there are frequent references to minute or miliary intracerebral aneurysms, which were believed by some

investigators to be small dissecting aneurysms. These tiny swellings are located in the small, and often thread-like, intracerebral and meningeal arterioles. Indeed, Charcot and Bouchard[13] believed that these miliary aneurysms were responsible for most instances of cerebral hemorrhages. Recently this subject has been reviewed in another report by me.[14] If such miliary lesions ever were common, they certainly are rare today, for they are seldom observed or reported and their role in the development of cerebral hemorrhage is questionable.

SUMMARY

A dissecting aneurysm of the right middle cerebral artery occurred as an unfortunate complication of the surgical removal of a berry aneurysm in a 46-year-old woman. Thrombosis with occlusion of the artery resulted, causing massive infarction of a large part of the hemisphere.

A review of the reports of similar lesions disclosed their relative rarity. While the development of dissecting aneurysms of intracranial arteries is not common, it is possible that such lesions have often been overlooked or actually ignored because they were obscured by more obvious lesions such as thrombosis with infarction or massive hemorrhage.

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Effects of Heparin on Development of Atherosclerosis and Fatty Liver

A Report of Studies on Cholesterol-Fed Rabbits

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Intravenous administration of heparin is known to clear alimentary lipemia. It also may influence the deposition of lipids in various tissues and organs, among these being the coronary artery, aorta, liver, and possibly others. Thus, information concerning the effects of its administration upon the development of atherosclerosis and fatty liver would be of particular interest and importance.

Graham and co-workers [1] first reported that daily injection of heparin into cholesterol-fed rabbits appeared to retard or prevent the development of atherosclerosis. This finding was confirmed by Constantinides and co-workers [2] and by Horlick and Duff.[3] In the studies of the above-mentioned workers, 10 to 50 mg. of heparin per rabbit per day was administered.

It was shown by Block, Barker, and Mann [4] that in the majority of instances male atherosclerotic patients showed much less clearing of alimentary lipemia following a small dose of heparin than did normal subjects. The authors indicated that their findings appeared to show a true relationship between atherosclerosis and resistance to clearing of lipemic plasma by heparin. Oliver and Boyd [5] found that only 31% of the alimentary lipemia was cleared after the administration of heparin to a group of subjects with coronary artery disease as compared with 64% in a control group. However, there is a slight overlap of individual values for clearing between the disease group and the controls. They commented that this test of clearing activity as an adjuvant to the diagnosis of coronary artery disease was of limited value.

Little information is available concerning the effect of heparin on the development of fatty liver. Kessler and Meng [6] found that daily intraperitoneal injection of heparin (1 mg/rat/day) into choline-deficient cholesterol-fed male rats produced a significant decrease in the content of liver fat. However, in the study of Constantinides and his co-workers [2] heparin failed to prevent the fatty infiltration of the liver in cholesterol-fed rabbits.

In view of our limited and incomplete knowledge of these problems, the present investigation was undertaken to study the following: (1) the effects of parenteral administration of small doses of heparin (2 mg/rabbit/day) on the development of atherosclerosis and fatty liver in cholesterol-fed rabbits; (2) the relationship between the incidence of atherosclerosis and the ability of postheparin plasma of these animals to clear the turbidity of neutral fat emulsion in vitro.

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From the Department of Physiology, Vanderbilt University School of Medicine.

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MATERIALS AND METHODS

Forty-five male albino rabbits weighing 1.5 to 3.0 kg. were used in this study. All animals were fed Purina Rabbit Checkers ad libitum during a 10- to 14-day control period. Immediately following this period, 0.5 gm. of cholesterol mixed with 2.5 gm. of cottonseed oil and 0.25 gm. of oleic acid was given by force-feeding at 9 a. m. and 2 p. m. every day. Of the 45 rabbits, 21 received intraperitoneal injections of 1.0 mg. of heparin * twice a day one and one-half to two hours after cholesterol feedings, while the other 24 rabbits received injections of saline intraperitoneally. The experiments were carried out for eight to ten weeks.

Body weight was measured weekly under standardized conditions. Serum total cholesterol was determined every other week with the method described by Pearson and co-workers.[7] The heparin clearing test was carried out before and at intervals during cholesterol feeding, usually in the morning. The principle of the clearing test has been stated by Meng and co-workers,[8] and the modified procedure is briefly described as follows: Heparin, 0.05 mg/kg., was given intravenously into the marginal ear vein, and blood from the other ear vein was collected three to five minutes after heparin administration into an oxalated tube which was kept in ice water. Postheparin plasma, 0.5 ml., was added to a tube containing phosphate-acetate buffer (pH 6.5) and olive oil emulsion. The contents of the tube were mixed, and per cent of light transmittance was measured immediately at 700 mμ in a Coleman spectrometer. The tube was incubated for two hours at 37 C, after which per cent of light transmittance was again determined. Per cent clearing of the turbidity of the olive oil emulsion by postheparin plasma was obtained by subtracting the zero-hour reading from the two-hour reading. A similar test was also carried out with preheparin plasma. The per cent clearing attributed to heparin administration was calculated by subtracting the per cent clearing by preheparin plasma from that by postheparin plasma.

At autopsy the severity of aortic atherosclerosis was graded grossly as described by Horlick and Duff.[3] Microscopic examination of histologic sections of aortas stained with hematoxylin and eosin and with Sudan IV was also carried out.

The liver was weighed immediately after its removal from the animal. The presence or absence of fat in the liver was based on gross and microscopic examinations (hematoxylin and eosin and Sudan IV).

RESULTS

Incidence of Atherosclerosis.—Of the 24 rabbits which did not receive heparin, 18 showed aortic atherosclerotic lesions, the incidence therefore being 75%, while among the heparin-treated animals 9 of the 21 showed lesions, an incidence of 43%. Thus it appears that heparin did suppress the development of atherosclerosis in the cholesterol-fed rabbits. When the data were analyzed on the basis of the severity of atherosclerosis and the number of animals in each group, the following findings were revealed: In the nonheparinized group: no lesions, 6 rabbits; 1+, 15 rabbits, and 2+ or more, 3 rabbits. In the heparin-treated group: no lesions, 12 rabbits; 1+, 6 rabbits, and 2+ or more, 3 rabbits. The P value obtained from the χ^2 test is <0.06; thus the difference between the two groups is of borderline significance.

Serum Total Cholesterol.—As was expected, the average serum total cholesterol of the rabbits of both groups was elevated. Much to our surprise, the elevation of the serum total cholesterol of the heparin-treated group was significantly greater than that of

* Heparin sodium, 10 mg/ml. and 100 U. S. P. units/mg., was supplied by Dr. L. L. Coleman, The Upjohn Company, Kalamazoo, Mich.

TABLE 1.—*Average Serum Total Cholesterol of Cholesterol-Fed Rabbits*

Heparinized				Nonheparinized			
Diet	Week	Average Serum Total Cholesterol (Mg. %)	S.E. ±	Diet	Week	Average Serum Total Cholesterol (Mg. %)	S.E. ±
Basal *	1	73.2	3.6	Basal	1	76.9	3.5
	2	68.7	3.2		2	73.7	4.0
Basal + cholesterol	1	297.2	31.6	Basal + cholesterol	2	214.9	22.2
	3	504.5	40.2		4	311.2	35.6
	5	681.5	51.5		6	342.1	38.7
	8	421.3	93.2		8	395.1	50.5

* Purina Rabbit Checkers.

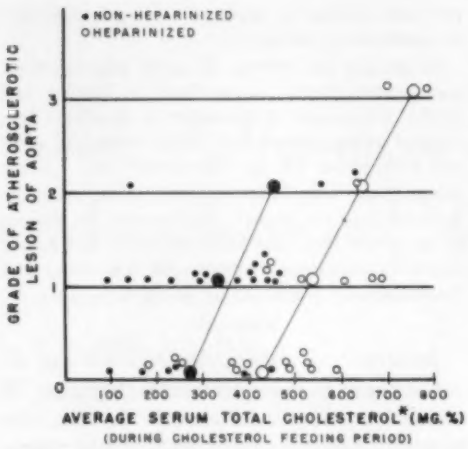

Fig. 1.—Average serum total cholesterol of individual rabbits; large black dots, mean of average serum total cholesterol of individual rabbits of nonheparinized group; large white circles, mean of average serum total cholesterol of individual rabbits of heparinized group.

the nontreated group, although it declined and reached the same level as that of the nontreated group toward the end of the experiment (Table 1).

Relationship Between the Severity of Aortic Atherosclerosis and Serum Total Cholesterol.—It can be seen in Figure 1 that the severity of aortic atherosclerosis bears a direct relationship to the serum total cholesterol level—the higher the serum total cholesterol, the severer the aortic lesions.

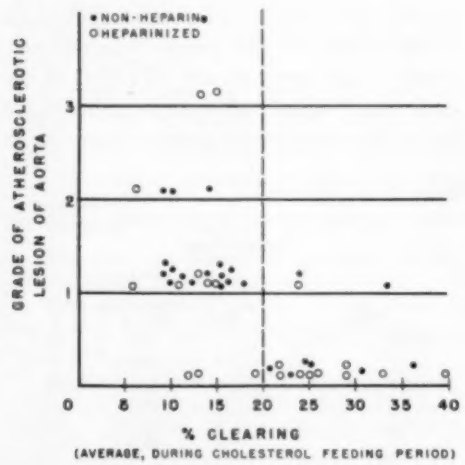

Figure 2

This is true in both groups of animals, regardless of heparin treatment.

Relationship Between the Incidence of Aortic Atherosclerosis and the Clearing Activity of Postheparin Plasma.—The relationship between the presence or absence of aortic atherosclerosis and the ability of postheparin plasma of cholesterol-fed rabbits to clear the

Table 2.—*Effect of Heparin on Liver Weight and Fat Accumulation*

Rabbit No.	Group	Body Weight (At Time of Sacrifice) (Kg.)	Liver		Accumulation of Fat
			Weight	% Body Weight	
C 2	Heparin	1.98	50.5	2.58	—
C 3	Heparin	2.59	69.5	2.68	+
C 5	Heparin	2.53	65.0	2.57	—
C 8	Heparin	2.26	72.5	3.21	—
C 9	Heparin	2.70	64.6	2.39	—
C 10	Heparin	2.31	61.2	2.65	+
C 12	Heparin	2.56	62.0	2.42	—
C 13	Heparin	2.92	60.5	2.07	—
C 14	Heparin	2.84	69.9	2.46	—
C 15	Heparin	2.79	88.5	2.99	—
C 17	Heparin	2.37	73.5	3.09	—
C 19	Heparin	2.40	57.0	2.38	—
	Mean ± S.E.	2.52 ± 0.08	65.8 ± 2.5	2.62 ± 0.09	16.7%
E 1	Nonheparin	2.35	79.0	3.36	—
E 2	Nonheparin	2.82	85.0	3.01	+
E 3	Nonheparin	2.50	80.0	3.20	+
E 4	Nonheparin	2.08	55.1	2.65	+
E 5	Nonheparin	2.66	80.2	3.02	+
E 7	Nonheparin	2.40	76.3	3.18	+
E 10	Nonheparin	2.70	90.0	3.33	+
E 12	Nonheparin	1.80	69.3	3.85	—
E 13	Nonheparin	2.54	90.0	3.54	+
E 14	Nonheparin	2.78	87.5	3.15	—
E 15	Nonheparin	2.74	72.5	2.65	—
	Mean ± S.E.	2.49 ± 0.10	78.6 ± 3.1	3.18 ± 0.11	64%
	P value	Not sig.	<0.01	<0.001	

turbidity of neutral fat emulsion in vitro is shown in Figure 2. With a vertical line drawn at 20% clearing, it can be seen that of the 27 animals to the left of the line only 3 did not show aortic atherosclerosis; the other 24 rabbits, which had an average clearing of less than 20%, had lesions. It should be pointed out that the three animals which did not show aortic atherosclerosis were all from the heparin-treated group. In contrast to these findings, of the 18 animals to the right of the vertical line only 3 showed aortic

atherosclerosis. The remaining 15 animals did not show any lesions.

It can also be seen in Figure 2 that of the 18 rabbits to the right of the vertical line 10 were heparin-treated and 8 were nontreated. The relationship between the two groups of animals to the left of the vertical line was reversed; namely, 11 of the 27 animals were heparin-treated and 16 were nontreated.

Heparin Administration and the Incidence of Fatty Liver.—Only data obtained from 23 rabbits (12 heparin-treated and 11 nontreated) are shown in Table 2. It can be seen that the difference in body weight between the heparin-treated and nontreated animals was not statistically significant. However, the mean liver weight of nontreated rabbits was 12.8 gm. more than that of the heparin-treated group; the P value was <0.01, which is significant. The difference in liver weight between the two groups of animals was even more significant when compared in terms of per cent of body weight; the P value was <0.001.

COMMENT

Our results confirmed the observations of Graham and co-workers,[1] Constantinides and co-workers,[2] and Horlick and Duff [3] that daily injection of heparin into cholesterol-fed rabbits prevented or retarded the development of aortic atherosclerosis. However, the difference in the incidence and severity of the aortic lesions between the heparin-treated and nontreated animals is not so great as that reported by Horlick and Duff.[3] This may be due to the fact that only 2 mg. of heparin per rabbit per day was given in the present study. This is only 4% to 20% of the dose administered by other investigators.†

The considerably greater elevation in serum total cholesterol in the heparin-treated animals than that in controls observed in our study is at variance with the finding of Constantinides and co-workers.[2] Basu and Stewart [9] also found that administration of heparin produced a decrease in plasma cholesterol levels, particularly when hypercholesteremia was present. Friedman and Byers [10] demonstrated that administration of heparin was capable of reducing serum cholesterol in rats rendered hypercholesteremic by the injection of an oxyethylated tertiary-octylphenol-formaldehyde polymer (Triton WR-1339). However, studies of Horlick and Duff,[3] which were similar to our investigation, showed no consistent difference in serum total cholesterol between the heparin-treated and nontreated rabbits.

The results shown in Figure 1 suggest that a higher serum total cholesterol level is necessary to produce the same degree of aortic atherosclerosis if heparin is administered.

A reversed relationship between the incidence of aortic atherosclerosis and clearing activity of postheparin plasma of the cholesterol-fed rabbits is demonstrated in the present study. In this study we confirm the observations of Block and co-workers [4] and of Oliver and Boyd [5] in man and suggest that the heparin clearing test might aid in the diagnosis of atherosclerosis. The small percentage of supposedly normal subjects, animal and man, whose individual values for clearing overlapped those of the disease group might have been potentially atheromatous. However, further studies are necessary before any conclusions can be made. It may be suggested here that the in vitro heparin clearing test may be more advantageous than the in vivo method for clearing of alimentary lipemia, from the standpoint of both technical simplicity and reliability of results.

The results shown in Figure 2 indicate that the difference in clearing of the turbidity of neutral fat emulsion by postheparin plasma between the heparin-treated and nontreated rabbits is probably not significant. It seems that although the exogenous supply of heparin is capable of preventing or retarding the development of aortic atherosclerosis, it cannot improve the ability of postheparin plasma to clear.

The effects of heparin on liver weight and the incidence of fatty liver of cholesterol-

† References 1, 2, and 3.

fed rabbits are of interest. Unfortunately, chemical analyses for liver lipids and fractions and liver nitrogen and water content were not carried out in the present study. Further work in this regard is necessary to demonstrate the possible role of heparin as a lipotropic agent.

SUMMARY

Daily intraperitoneal injection of 2 mg. of heparin per rabbit per day into cholesterol-fed rabbits suppressed the development of aortic atherosclerosis by approximately 40%, as judged by gross grading methods.

The ability of postheparin plasma to clear the turbidity of neutral fat emulsion (heparin clearing test) was determined. In 15 of the 18 cholesterol-fed rabbits whose postheparin plasma produced 20% clearing or more, aortic atherosclerosis was not observed. On the other hand, 24 of the 27 rabbits whose postheparin plasma produced less than 20% clearing had lesions.

Daily intraperitoneal administration of 2 mg. of heparin per rabbit per day into cholesterol-fed rabbits prevented the accumulation of fat in the liver by approximately 60%, as judged by gross and microscopic examinations.

Dr. Margaret P. Martin and Mr. Edwin Bridgeforth, Department of Preventive Medicine, Vanderbilt University School of Medicine, did the statistical analysis of the data presented in this paper. Dr. John L. Shapiro, Department of Pathology, Vanderbilt University School of Medicine, interpreted the histologic sections.

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Spontaneous Salivary Gland Virus Disease in Chimpanzees

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Cytological evidence of salivary gland virus disease characterized by large intranuclear and intracytoplasmic inclusion bodies has been found in human beings,* monkeys,[4] guinea pigs,[5] mice,[6] rats,[6] hamsters,[6] and moles.[7] Under natural and experimental conditions, the virus has manifested absolute species specificity and a low degree of virulence.[8] The natural resistance displayed by the human fetus and young infant is notably low, for not only are pathognomonic inclusion bodies contained within the salivary glands of 10% to 30% of unselected postmortem cases [9] but widespread involvement of many organs by disseminated salivary gland virus disease or cytomegalic inclusion disease has been observed repeatedly.† By contrast, the infection has been rarely encountered in older children and adult human beings.‡

The study reported here deals with the occurrence of spontaneous salivary gland virus infection in chimpanzees. The high incidence of the disease, its occurrence in disseminated form, and its presence in mature as well as in young animals suggest that this laboratory primate possesses a low degree of natural resistance to infection by an unidentified strain of the salivary gland virus. The findings provide a basis for an investigation of the susceptibility of the chimpanzee to the human strain of salivary gland virus, to be reported later.

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This study was conducted at the Radiobiological Laboratory of The University of Texas and the United States Air Force, Austin, Texas; supported in part by funds provided under Contract AF 18(600)-165 with the U. S. A. F. School of Aviation Medicine, Randolph Field, Texas.

* References 1 through 3.

† References 10 and 11.

‡ References 12 through 14.

MATERIALS AND METHODS

The observations herein described are derived from detailed gross and microscopic postmortem examinations performed on 12 chimpanzees.

Chimpanzees.—The subjects were male animals with estimated ages between 3 and 17 years, and weights from 20 to 120 lb. (9.1 to 54.4 kg.). They were procured from the Chase Wild Animal Farm, Egypt, Mass., and from the Trefflich Bird and Animal Company, New York. All animals were maintained on well-balanced diets under close veterinary surveillance.

Histological Techniques.—The hematoxylin and eosin stain was used routinely on formalin-fixed, paraffin-embedded tissues. Other histological techniques, Shorr's trichrome stain for inclusion bodies, Masson's trichrome, Unna's, and Giemsa's stains, were employed on selected portions of tissue.

REPORT OF CASES

Spot.—A male chimpanzee, age 4 to 5 years, was housed at the laboratory for 12 months; during this time a weight gain of 19 lb. (8.6 kg.) occurred without clinical manifestations of illness. Death followed the administration of a standard anesthetic dose of pentobarbital (Nembutal).

The postmortem examination disclosed disseminated salivary gland virus disease, with characteristic intranuclear inclusion bodies within markedly enlarged parenchymal cells of the parotid and submaxillary glands, the cortex of each adrenal gland, and the myocardium. A detailed description of the cytological features is given in a later section of this report.

Tony.—A 17-year-old, 120 lb. (54.4 kg.) male chimpanzee had been maintained at the laboratory

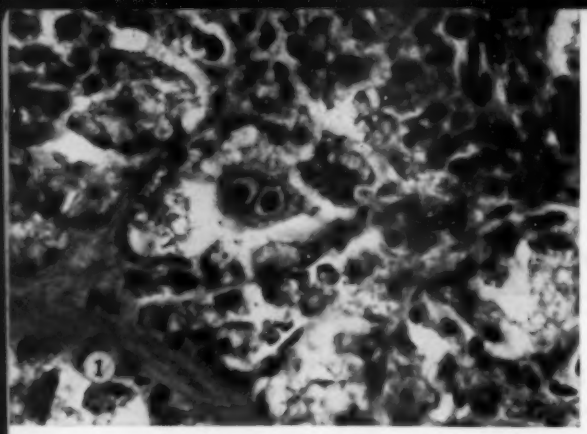


Fig. 1.—Section of the parotid gland of a chimpanzee (Spot) containing a binucleated, cytomegalic epithelial cell that presents the pathognomonic features of salivary gland virus disease. The large acidophilic intranuclear inclusion bodies are separated from the marginated chromatin material and the nuclear membranes by conspicuous halos. Hematoxylin and eosin; reduced ¼ from mag. × 580.

for 13 months and had been in good health until 2 weeks prior to death. The terminal illness was characterized by diarrhea, anorexia, and lethargy.

Disseminated salivary gland virus disease was disclosed on postmortem examination. Evident at necropsy were many 2 to 4 mm. light yellow areas of malacia throughout the cortex of each adrenal gland. Within these regions, as proved on histological section, there were numerous enlarged parenchymal cells with one or several nuclei containing huge intranuclear inclusion bodies. Many of the cortical cells had undergone necrosis, and multitudes of neutrophilic leucocytes were present around about them. Many sections of each submaxillary gland failed to contain demonstrable inclusion-bearing cells.

Sad Sack.—A 4-year-old, 26 lb. (11.8 kg.) male chimpanzee was under observation at the laboratory for five months, during which time the animal frequently manifested signs of an upper respiratory infection. The terminal illness, of two months' duration, was characterized by persistent diarrhea.

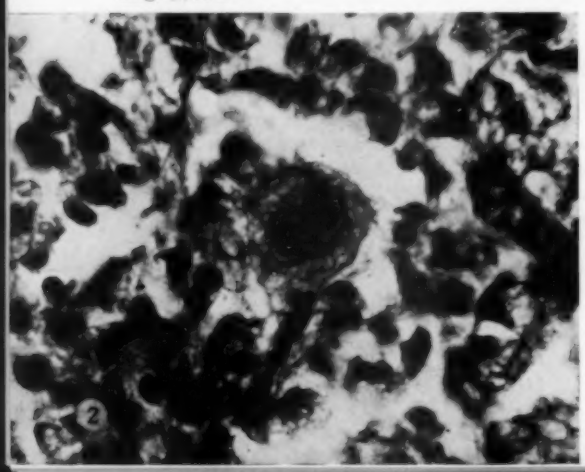
The postmortem examination showed disseminated salivary gland virus disease. Characteristic intranuclear inclusion bodies were present within enlarged acinar cells of the submaxillary glands and the cortical cells of each adrenal gland.

The finding of disseminated salivary gland virus disease in 3 of 12 chimpanzees was broadened by the presence of latent viral infections of the submaxillary glands in an additional 5 animals, as proved histolog-

Incidence and Distribution of Spontaneous Salivary Gland Virus Infection in Chimpanzees

Animal	Sex	Wt., in Lb.	Estimated Age, Yr.	Organs Involved by Salivary Gland Virus	Other Pathological Conditions
Spot	M	37	4 to 5	Rt. and lt. parotid and submaxillary glands, cortex of rt. and lt. adrenal glands and myocardium	Balantidium coli infestation of colon without ulceration
Tony	M	120	17	Cortex of rt. and lt. adrenal glands	Strongyloides infestation of small and large bowel with extensive ulceration
Sad Sack	M	26	4	Rt. and lt. submaxillary glands and cortex of rt. and lt. adrenal glands	Balantidium coli infestation of colon
Joey	M	21	3 to 4	Rt. and lt. submaxillary glands	Bronchopneumonia
Sleepy	M	20	3 to 4	Rt. and lt. submaxillary glands	Bronchopneumonia
Buck	M	36	4 to 5	Rt. submaxillary gland	Amebiasis with ulcerative colitis
Lover	M	24	4	Lt. submaxillary gland	Amebiasis with ulcerative colitis and hepatic abscesses
Gamma	M	42	5	Lt. submaxillary gland	Amebiasis with ulcerative colitis
Beau	M	44	5	None	Pulmonary tuberculosis
Mike	M	40	5	None	Amebiasis with ulcerative colitis
George	M	41	4 to 5	None	Pulmonary tuberculosis
Pat	M	44	5	None	Amebiasis with ulcerative colitis; bronchopneumonia

Fig. 2.—High magnification of a typical inclusion-bearing cell of the parotid gland. The cytoplasm is vacuolated and contains small basophilic granules. Hematoxylin and eosin; reduced ¼ from mag. × 950.



ically by the demonstration of cytomegaly with pathognomonic intranuclear inclusion bodies. The pertinent findings in all animals are summarized in the accompanying Table.

MORPHOLOGICAL CHARACTERISTICS

Inclusion-Bearing Cells and the Inclusion Bodies.—The affected cells were notably similar in appearance in all sites and organs. They were several times enlarged above the size of the normal parenchymal elements and measured up to 30μ to 60μ in greatest dimension. More than half of the cells were binucleated, while in approximately one-fourth there were three to six nuclei, each measuring 10μ to 20μ across. The intranuclear inclusion bodies were consistently uniform in size, having an average maximal dimension of 15μ. Each occupied the greater portion of the nucleus and stained weakly acidophilic with a uniform finely granular texture. Almost without exception, a clear halo separated the intranuclear mass from the nuclear membrane. Within this clear zone there were many small individual and clumped chromatin-like basophilic fragments lying adjacent to the inner aspect of the nuclear membrane (Figs. 1 and 2).

The cytoplasm of the inclusion-bearing cells was regularly abundant and occasionally contained basophilic granules in areas of rarefaction (Fig. 2). In general, these granules were smaller and less distinctive than the intracytoplasmic inclusion bodies in human beings.

Submaxillary and Parotid Adenitis Due to the Salivary Gland Virus.—Within the histological sections of the diseased salivary glands the number of inclusion-bearing cells varied from one to several per square centimeter of tissue. Most frequently they occupied the lumen of the acinus, being either unattached or adherent by narrow strands of cytoplasm to the basement membrane or to a nonaffected acinar cell. They were rarely found within ducts. Many small focal aggregates of lymphocytes and plasma cells were present throughout the glandular

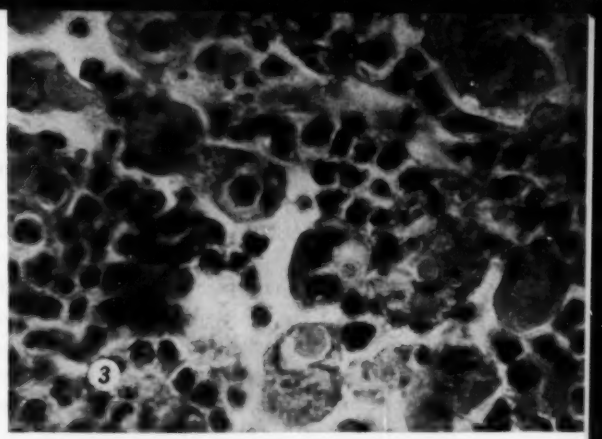
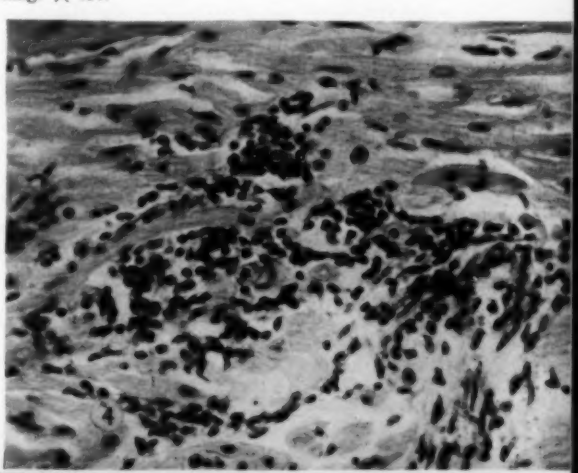


Fig. 3.—An area of necrosis with dense leucocytic infiltration in the adrenal cortex of a chimpanzee (Tony). Many parenchymal cells are enlarged and contain characteristic intranuclear inclusion bodies. Hematoxylin and eosin; reduced ¼ from mag. × 900.

parenchyma; however, few inclusion-bearing cells were associated with these inflammatory nodules.

Adrenocortical Adenitis Due to Salivary Gland Virus.—Pathognomonic intranuclear inclusion bodies were present in the cortex of the adrenal glands of each of the three chimpanzees with disseminated disease. In one animal (Tony) the involvement was extensive and was characterized by areas of dense leucocytic infiltration and necrosis that in some places extended through the entire width of the cortex. In and about these regions there were numerous enlarged cortical cells with conspicuous intranuclear inclusion bodies. By contrast, the pathological process in two animals (Spot and Sad Sack) was typified by small focal lymphocytic and plasmocytic infiltrations with minimal necrosis

Fig. 4.—One of many focal areas of inflammation in the myocardium of a chimpanzee (Spot), characterized by a single intranuclear inclusion body in a myocardial cell, early necrosis of the sarcoplasm, and a dense infiltration of lymphocytes and plasma cells. Hematoxylin and eosin; reduced ¼ from mag. × 450.



and few inclusion-bearing parenchymal cells (Fig. 3).

Myocarditis Due to Salivary Gland Virus.—One animal (Spot) showed an extensive myocarditis characterized by numerous focal collections of lymphocytes and plasma cells with patchy myocardial necrosis in equal concentrations throughout all depths of the myocardium and in the walls of each of the four chambers. Several typical intranuclear inclusion bodies were present within enlarged myocardial cells, the cytoplasm of which was swollen and had indistinct or no cross striational structure (Fig. 4).

COMMENT

The diagnosis of salivary gland virus disease was clearly established in 8 of 12 chimpanzees by the presence of pathognomonic intranuclear inclusion bodies with cytomegaly. The cytological characteristics of the spontaneous disease in this primate were essentially identical to those that have frequently been observed in human beings.[3] The visceral distribution of the disease in its disseminated form in the near-mature and mature chimpanzees resembled that in the human adult cases. The adrenal glands have not been frequently involved by infections that have occurred in the human fetus or during the newborn period[10] but have been strikingly altered in adult cases § and were similarly affected in the chimpanzees. In general, myocarditis has been an unusual manifestation of salivary gland virus disease in human beings. It has been observed with highest incidence in the adult cases and has been regularly characterized by cytological features that were indistinguishable from those present in the chimpanzee. The kidneys, lungs, and liver are frequently involved in human beings; they were normal in the small group of subhuman primates.

Several findings provide evidence to suggest that the chimpanzee might possess an unusually high degree of susceptibility to infection by the salivary gland virus. As regards incidence, with the possible exception of certain selected strains of guinea pigs and mice, the spontaneous disease is not known to attain a frequency in laboratory animals comparable with that in this primate colony. Dissemination characterizes the disease in the human fetus and is viewed as a manifestation of low host resistance; in like manner, its presence in 25% of the chimpanzees most probably connotes a high degree of susceptibility. Many observations make it clear that in human beings and laboratory animals there is an increase in natural resistance with age beyond the fetal and newborn periods. Thus, the presence of infection in 8 of 12 chimpanzees that were near or at maturity makes it seem likely that an even greater degree of susceptibility might exist in this primate during the early periods of life.

Several questions are opened by these findings. Is the spontaneous disease in the chimpanzee due to infection by the human strain or another strain of salivary gland virus? If the latter, then what is the degree of natural resistance of this primate to the human strain of the virus?

SUMMARY

Salivary gland virus disease with pathognomonic intranuclear inclusion bodies was present in 8 (67%) of 12 chimpanzees. The disease was disseminated in three animals (25%) and involved salivary glands, the cortex of each adrenal gland, and, in one case, the myocardium as well.

The incidence of the disease, its occurrence in disseminated form, and its presence in adult as well as young chimpanzees indicate a low degree of natural resistance in this primate to infection by the salivary gland virus.

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Gastric Neurofibromas Simulating Granulomas

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Recently Vanek,[1] Helwig and Ranier,[2] and Bullock and Moran [3] described certain polypoid gastric lesions composed of mesenchymal elements and infiltrated by lymphocytes and eosinophiles to which they applied the terms "gastric submucosal granulomas with eosinophilic infiltration" and "inflammatory fibroid polyps."

Three identical lesions were encountered in the surgical pathologic laboratory at the Cedars of Lebanon Hospital in recent years and were clearly neurogenic neoplasms. It seemed worth while to report these three cases in order to encourage other workers to present their views and help decide whether a new entity has been identified or whether such growths are already well-recognized lesions.

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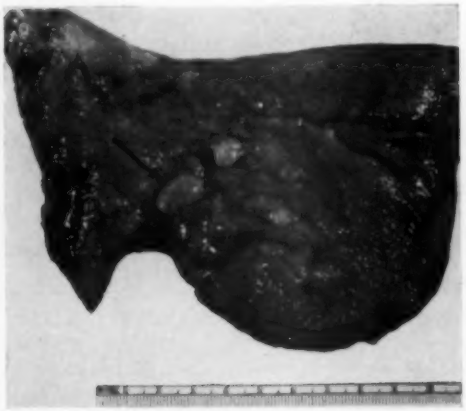
From the Division of Laboratories, Cedars of Lebanon Hospital. Dr. Sargent is a Trainee, National Cancer Institute.

The surgical resections were performed by Drs. Max Bay, Louis Sperling, and David State; photography by Dale Gillette.

Fig. 1 (Case 1).—Resected gastric segment containing several adenomas in addition to the neurofibroma (arrow).



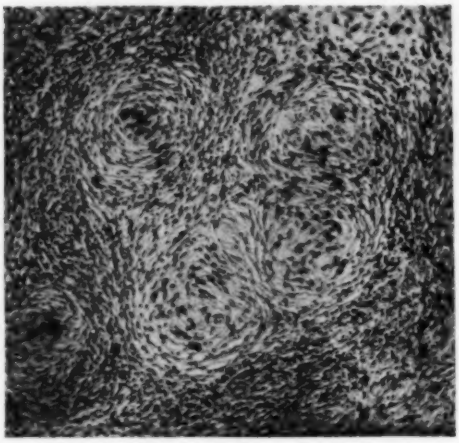


Fig. 2 (Case 1).—Microscopic view of tumor suggesting differentiation of neural structures; reduced ⅓ from mag. × 180.

REPORT OF CASES

Case 1.—A 72-year-old white man was admitted to the hospital for medical treatment of cholecystitis. After subsidence of symptoms radiologic examination of the stomach showed multiple gastric polypoid lesions. At operation a portion of the stomach containing several antral and pyloric polyps was resected, followed by a gastrojejunostomy and cholecystectomy.

The resected portion of the stomach contained several polypoid masses on the posterior wall (Fig. 1). The two largest measured 2.0 cm. and 1.5 cm., respectively; three smaller masses were also present. Three of the lesions were adenomas or hamartomas. The next to the largest was composed of spindle cells, many of which arranged themselves in tiny whorled bodies (Fig. 2) recalling the end-organs commonly seen in certain neural tumors. Bodian stain revealed many fibers (Fig. 3). The extensive cellular infiltrate contained many eosinophiles.

Case 2.—A 46-year-old white woman was admitted to the hospital with a three-year history of

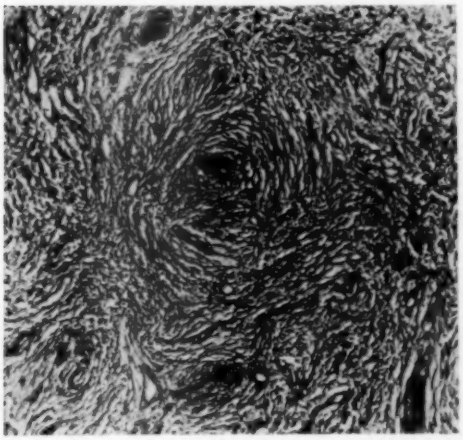
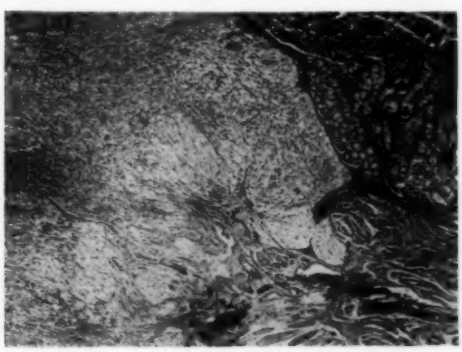


Fig. 3 (Case 1).—Bodian stain revealing many impregnated fibers; reduced ⅓ from mag. × 100.

weekly episodes of postprandial epigastric heaviness and indigestion, occasionally accompanied by nausea and vomiting. Radiologic examination of the stomach was suggestive but not diagnostic of an antral polyp. At operation a pedunculated polypoid lesion which could be pushed into the duodenum was seen arising from the middle portion of the antrum adjacent to the pyloric ring. This was resected together with a surrounding strip of stomach. Further exploration revealed two adenomas in the sigmoid, which were resected.

The specimen of stomach contained a 1.5×0.8 cm. polypoid lesion covered with mucosa, the tip of which was slightly hemorrhagic and granular. Microscopically the lesion showed spindle-cell elements arranged in a fibrillar network in interlacing bundles. A large part of the cellular infiltrate consisted of dense eosinophilic accumulations. The mucosal covering showed focal areas of both hyperplasia and atrophy. The base of the lesion was within the submucosa and seemed to be continuous with large nerve bundles (Fig. 4). Silver impregnation revealed many fibers.

Fig. 4 (Case 2).—Microscopic view of neurofibroma showing the base of the lesion within the submucosa and connections with nerve bundles; reduced ⅓ from mag. × 30.



Case 3.—A 72-year-old white man entered the hospital with a three-week history of sharp pain across the lower chest. Radiologic examination of the stomach showed a pyloric polyp herniating into the duodenal bulb. At operation the mass was seen to arise from the gastric mucosa along the lesser curvature of the stomach 2.5 cm. proximal to the pylorus. The specimen consisted of a 2×2×1 cm. ovoid mass completely covered with pink-tan mucosa. Microscopically the lesion showed interlacing fascicles of fibrillary and spindle-cell elements with an inflammatory cell infiltrate. The groupings were less definite than in Case 1 but

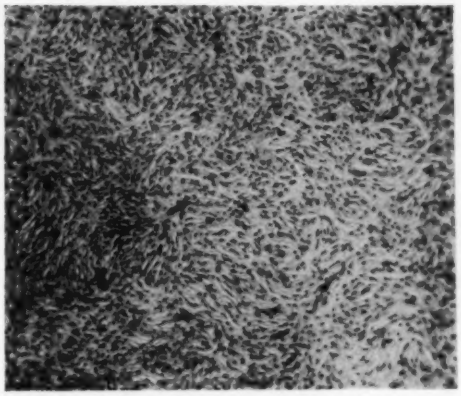
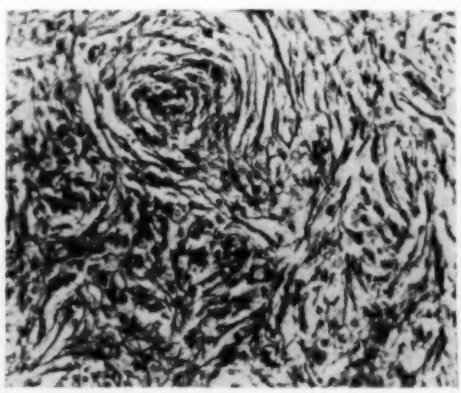


Fig. 5 (Case 3).—Microscopic view of tumor showing interlacing bundles of spindle cells; reduced ⅓ from mag. × 180.

Fig. 6 (Case 3).—Many fibers are revealed by silver impregnation methods; reduced ⅓ from mag. × 360.



still suggested organoid differentiation (Fig. 5). Silver preparations impregnated many fibers (Fig. 6). The overlying mucosa showed intestinal metaplasia and chronic inflammation.

COMMENT

Although we recognize that the differential diagnosis of spindle-cell tumors in general and the identification of neurogenic growths in particular are based oftener on the climates of opinion in various laboratories than on demonstrable and generally accepted histologic criteria, it has seemed to us that the growths under discussion would be considered neurofibromas in most laboratories. The formation of differentiated organoid structures in Case 1 and the relationship to nerves in Case 2 would lead to a diagnosis of neurofibroma without question if the tumors were encountered in, for example, subcutaneous tissue instead of the stomach. The significance of argyrophile fibers in these lesions is debatable.

The inflammatory cell infiltration and the eosinophilia are largely responsible for the view that the lesions are essentially granulomatous and inflammatory. However, the stromal patterns are not at all suggestive of granulation tissue or cicatricial fibrosis. The suggested comparison with granulomatous prostatitis [3] cannot be accepted, since the illustrative photomicrograph suggests a hyperplastic stromal nodule. Similarly, the granulomas of the stomach described by Sherman and Moran [4] present a completely different appearance.

SUMMARY

Three polypoid neurofibromas of the stomach are reported. It is suggested that secondary inflammation in such lesions may obscure the underlying growth and lead to the erroneous diagnosis of an inflammatory process.

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Demonstration of All Connective Tissue Elements in a Single Section

Pentachrome Stains

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In current studies of arteriosclerosis, collagen diseases, and experimental lesions of hypersensitivity, it was essential to have a staining method which would define all the elements of connective tissue, including the acid mucopolysaccharides of the ground and cementing substances and the alteration termed fibrinoid degeneration or necrosis, in a single section.

Excellent methods are available for the demonstration of connective tissue fibers and membranes, but the ground substance in which these are embedded is usually poorly demonstrated. However, methods are now available which will stain the acid mucopolysaccharides of ground and cementing substances. Of these latter methods, Hale's [4] dialyzed iron and Steedman's [12] Alcian blue techniques can be combined with other connective tissue stains. Numerous combinations are possible. Rinehart and Abul-Haj [9] have suggested a combination of dialyzed iron with cochineal and picrofuchsin. Of all combinations we found the one to be described the most satisfactory. This is a combination of Weigert's iron-hematoxylin for nuclei, woodstain scarlet-acid fuchsin or orange G for cytoplasm, resorcin-fuchsin for elastic tissue, saffron or diphenyl fast red for collagen, and Alcian blue for the ground and cementing substances.

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From the Department of Pathology, Queen's University. Carried out with the aid of a grant from the National Research Council of Canada.

PREPARATION OF STAINS

1. *Weigert's Iron-Hematoxylin.*—Solution A: Dissolve 1 gm. of hematoxylin in 100 ml. of 95% alcohol.

Solution B: To 95 ml. of distilled water add 4 ml. of 29% ferric chloride and 1 ml. of HCl (specific gravity 1.124). Mix equal parts before use. The mixture can be used from two to three weeks. It is important for these staining methods to prepare Solution B with HCl of specific gravity 1.124 (the "*offizielle Salzäure*") and not with concentrated HCl, as stated in some textbooks.* It is prepared by adding 50 ml. of distilled water to 100 ml. of concentrated HCl (specific gravity 1.19).[10]

2. *Weigert-Hart Resorcin-Fuchsin.*—To 94 ml. of 70% alcohol add 5 ml. of Weigert's resorcin-fuchsin solution and 1 ml. of concentrated HCl.

3. *Woodstain Scarlet-Acid Fuchsin.*—Prepare a 0.1% solution of woodstain scarlet N. S. conc.† in 0.5% acetic acid, and a 0.1% solution of acid fuchsin in 0.5% acetic acid. Mix 8 parts of the woodstain scarlet solution with 2 parts acid fuchsin solution.

4. *Alcoholic Saffron.*—Prepare an alcoholic extract of saffron ‡ (Safran de Gatinais—Grübler) by placing 6 gm. in 100 ml. of absolute alcohol, corked tightly to prevent hydration, in an incubator at 58 C for 48 hours before use. The extraction can be repeated on the same saffron by prolonging it to 14 days. Keep airtight in a dark bottle.

* References 5 and 7.

† E. I. du Pont de Nemours and Company, Inc., Wilmington 98, Del.

‡ Purchased from O. C. Watzka and Company, 1012 Sherbrooke St. W., Montreal, Canada.

This solution is similar to the one recommended recently by Bencosme [1] for a routine trichrome stain. It is, however, somewhat more concentrated.

5. *Orange G.*—Dissolve 1 gm. of orange G in 100 ml. of distilled water, and add 0.1 ml. of acetic acid.

6. *Diphenyl Fast Red.*—Dissolve 1 gm. of diphenyl fast red 5BL supra I § in 100 ml. of distilled water, using moderate heat. Cool, filter, and add 1 gm. of orange G and 0.1 ml. of acetic acid. More recently we used also chlorantine fast red 5B conc.‖ with equally good results.

7. *Alcian Blue.*¶—Dissolve 1.0 gm. of Alcian blue 8GS in 100 ml. of distilled water, filter, and add 1.0 ml. of acetic acid. This solution has to be prepared freshly each time before use. (Alcian blue obtained from National Aniline Division can also be used, but the result is a much paler blue; this can be improved slightly by dissolving the dye in warm water.)

FIXATION, DEHYDRATION, CLEARING, AND EMBEDDING

The desired perfection of the stain is achieved only when proper attention has been paid to fixation, dehydration, clearing, and embedding.

The fixatives tried were 10% buffered formalin, 10% neutral formalin, 25% formalin, Bouin-acetic, Bouin-trichloroacetic, Brazil, Zenker, Helly, Spuler-Maximow, Zenker-formol (20% formol), Zenker-acetic-formol, Susa, sublimate, formol-sublimate, and formol-sublimate-acetic. Among all the fixatives tried, formol-sublimate-acetic (F.S.A.) gave the most satisfactory results for the fixation of connective tissue. It is prepared by adding to 80 ml. of distilled water 4 gm. of mercuric chloride, 20 ml. of formol (36% to 40% formaldehyde solution), and 5 ml. of acetic acid. The tissues (3 to 4 mm. thick) are fixed for 12 to 18 hours in this fixative. The staining of tissues fixed in formalin can be improved by postfixing the slides a few hours in the F.S.A. fixative. Bichromate-containing fixatives cannot be used; the tissues stain diffusely with Alcian blue.

Particular care was taken to achieve excellent dehydration, clearing, and embedding. Failure to duplicate results as described in this paper may be due to inadequate fixation, dehydration, etc. The importance of this fact is often not sufficiently appreciated, and dehydration, clearing, and embedding schedules similar to those described recently by Bencosme [1] are essential to proper staining and definition of structure.

STAINING PROCEDURE

PENTACHROME I

1. Bring sections through toluol and absolute alcohol into 1% alcoholic (95%) iodine for one minute, wash, bleach with 5% Na thiosulfate, wash for 10 minutes, and rinse with distilled water.

2. Stain for 15 to 30 minutes in the Alcian blue solution.

3. Wash for two to three minutes in running water.

4. Place into alkaline alcohol (pH over 8) for two hours, prepared by adding ammonium hydroxide to 95% alcohol. It will convert the Alcian blue into the insoluble pigment monastral fast blue.

5. Wash for 10 minutes in running water.

6. Rinse in 70% alcohol.

7. Place into the Weigert-Hart resorcin-fuchsin solution overnight (16 hours).

8. Wash in running water for 10 minutes, and rinse in distilled water.

9. Overstain nuclei in Weigert's iron-hematoxylin for 2 to 10 minutes. Differentiation will take place in the woodstain scarlet, which contains acetic acid.

§ Supplied by Geigy and Company, Ltd., Toronto, Canada. It can be purchased also from Geigy Dyestuffs, 89-91 Barclay St., New York 8.

‖ Supplied by Ciba, Aktiengesellschaft, Basel, Switzerland. It can also be obtained from Ciba, Ltd., Montreal, Canada.

¶ Alcian blue 8GS was furnished by Imperial Chemical Industries Ltd., Dyestuffs Division, Hexagon House, Blackley, Manchester 9, England. It can be purchased also from Hoffman & Co., Inc., 55 Canal St., Providence 1, R. I.

10. Wash in running water for 10 minutes, and rinse in distilled water.

11. Stain cytoplasm for five minutes in the woodstain scarlet-acid fuchsin solution.

12. Rinse in 0.5% acetic acid.

13. Differentiate in 5% phosphotungstic acid. Differentiate (approximately 10 to 20 minutes) to the point at which the collagen is pale pink and the ground substance, which is covered by the red, is bluish again.

14. Rinse in 0.5% acetic acid.

15. Rinse thoroughly in three changes of absolute alcohol. It is essential not to use low-grade alcohols. If low-grade alcohols are used, the cytoplasmic stains are dissolved out and the tissues will not take on the collagen stain, which is made up in absolute alcohol.

16. Stain with the alcoholic saffron solution for 5 to 15 minutes. It is important to use an airtight staining jar because there must be no hydration of the alcoholic saffron.

17. Three changes of absolute alcohol. The alcohol must not contain a trace of water.

18. Place into the first toluol, and examine slides. If the collagen is not sufficiently yellow, repeat the staining with the saffron.

19. Three changes of toluol or xylene.

20. Mount in Permount.#

Results:

1. Nuclei: black
2. Cytoplasm: red
3. Elastic fibers: dark purple to black
4. Collagen and some reticular fibers: yellow to greenish-yellow
5. Ground substance and some reticular fibers: blue to bluish-green; fibrinoid stains an intense red

Pentachrome II

Steps 1 to 8 as in Pentachrome I.

9. Place into the iron-hematoxylin solution for 2 to 10 minutes. In this the slides will be overstained. Differentiation will take place in the following solutions, which contain acetic acid.

10. Wash for 10 minutes in running water, and rinse with distilled water.

11. Stain for five minutes in the orange G solution, and rinse with 0.1% acetic acid.

12. Differentiate and mordant for at least 20 minutes in 5% phosphomolybdic acid (phosphotungstic acid cannot be used).

13. Rinse with 0.1% acetic acid.

14. Stain for 30 minutes in the diphenyl or chlorantine fast red solution.

15. Rinse in 0.1% acetic acid.

16. Dehydrate with absolute alcohols, clear, and mount.

Results:

1. Nuclei: black
2. Cytoplasm: yellow to orange-yellow
3. Elastic tissue: dark purple to black
4. Collagen and some reticular fibers: dark red
5. Ground substance and some reticular fibers: blue to bluish-green; fibrinoid stains orange-yellow

Comment

A few comments regarding the technique are pertinent. Alcian blue as a stain was introduced by Steedman.[12] He dissolved the dye in distilled water and stained up to 40 seconds. If the sections were kept longer in the staining solution, not only the mucins but all the tissues stained blue. Pearse [8] recommended it as a stain for acid mucopolysaccharides. It was employed for the latter purpose by Italian workers,[14] and more recently by Lison [6] and Gomori.[3] Lison used 0.5% acetic acid, and Gomori, 5% aluminum sulfate as a solvent. In both of these the dye is specific and there is no staining of the background, even if the slides are left in the stain for several hours. It is, however, essential to make up a fresh solution each time before use. Alcian blue probably stains acid mucopolysaccharides. The distribution corresponds, at least in cardiovascular structures, to the metachromasia seen with toluidine blue. Incubation with testicular hyaluron-

"Permount" is made by Fisher Scientific Company, Pittsburgh.

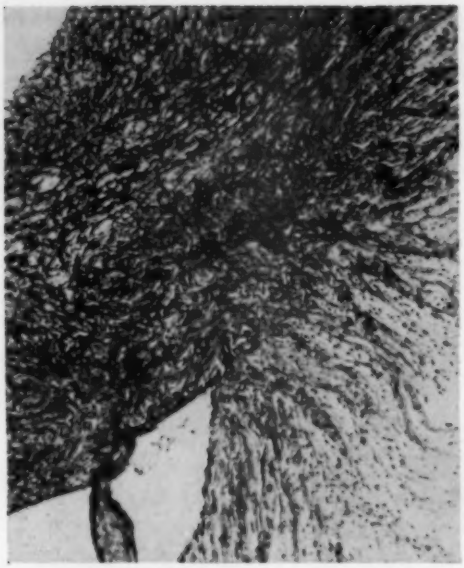
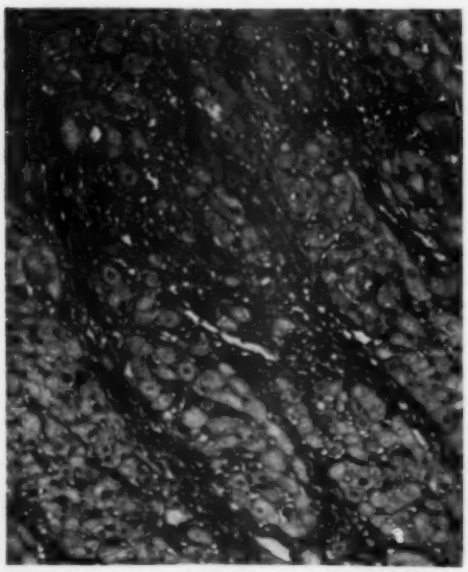


Fig. 1.—Human mitral valve. Nuclei are black, myocardium in right lower corner is red (pale gray in the illustration), ground substance between elastic and collagen fibers blue (dark gray to black), collagen yellow to greenish-yellow (medium gray), and elastic fibers are black. Fixed in F. S. A. Pentachrome I stain; × 80.

Fig. 2.—Myocardium of rabbit sensitized to horse serum. To left of center an area of recent muscle loss with marked increase of intermuscular Alcian blue-positive partly homogeneous and partly fibrillar connective tissue. Muscle yellow, nuclei black, and collagen dark red (black in the print). Fixed in F. S. A. Pentachrome II stain; × 260.



idase* impaired the staining of heart valves, aorta, and bronchial cartilage. A comparison with dialyzed iron shows that certain Alcian blue-positive structures which become negative upon incubation with hyaluronidase do not stain at all with the Hale technique. Experiments to further evaluate the histochemical specificity of Alcian blue for acid mucopolysaccharides are in progress.

The hematoxylin has to be made up with hydrochloric acid of specific gravity 1.124, as suggested originally by Weigert, in order

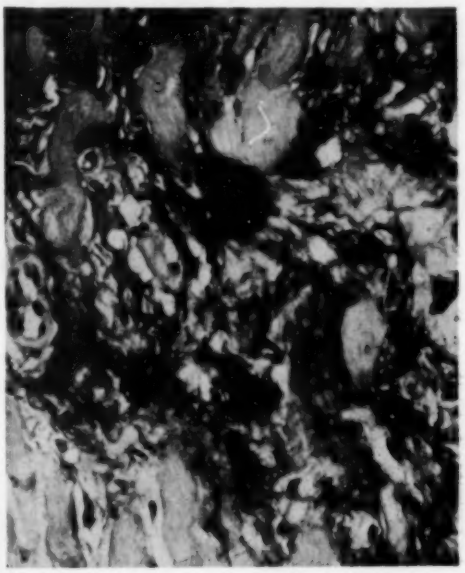


Fig. 3.—Myocardium of sensitized rabbit, early acellular sclerosis. Remaining myocardial fibers (top and bottom of photomicrograph) yellow. Most of section represents area of muscle loss, with replacement by Alcian blue-positive material (gray). Acellular collagenization seen in red (black) is gradually replacing Alcian blue-positive ground substance. Fixed in F. S. A. Pentachrome II stain; × 325.

to obtain dark overstained nuclei, which are later differentiated automatically in the acetic acid containing woodstain scarlet, orange G, and diphenyl fast red. The resulting nuclear stain is very intense and sharp (Fig. 5).

* Hyaluronidase (Wydase) was supplied by J. Wyeth and Brothers (Canada) Ltd., Walkerville, Ont.

The ratio of woodstain scarlet to acid fuchsin of 8:2 was found most satisfactory. If more contrast between the yellow collagen and the cytoplasm is desired, the ratio of 7:3 can be used. In Pentachrome II only phosphomolybdic acid can be used for mordanting; phosphotungstic acid differentiation and mordanting are followed by inadequate staining with the red collagen stain.

RESULTS AND CONCLUSIONS

As stated in the introduction, the methods described were developed during a search for an appropriate connective tissue stain for the study of lesions occurring in these tissues. Thus far, the methods have been applied to a variety of normal structures, mainly of the cardiovascular system. They have also been used extensively in the study of experimental hypersensitivity, the collagen diseases, and arteriosclerosis, and they have proved very useful in the study of mesodermal tumors. The interpretation and findings in these studies will be published at a later date. A few examples of the application of the methods to the studies referred to and their results will be presented briefly.

Fig. 4.—Normal human aorta. In this section the distribution, quantity, and relation of the Alcian blue-positive ground substance (dark gray) to the smooth muscle, collagen, and elastic fibers (pale gray) can be seen. Elastic stain was omitted. Fixed in F. S. A. Pentachrome I stain; × 350.

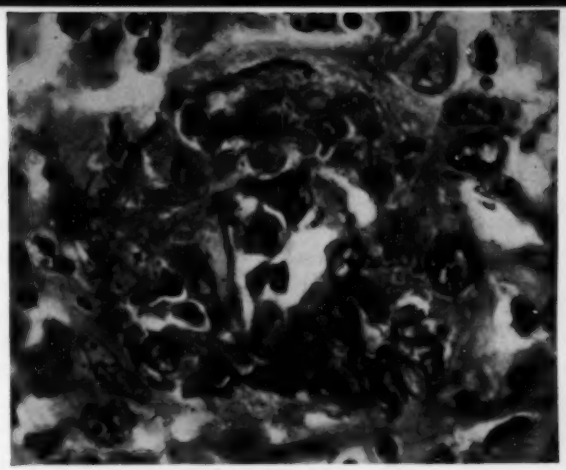


Fig. 5.—Artery of striated muscle, periarteritis nodosa. Thickened intima inside of fragmented internal elastic lamina. Fibrinoid (dark gray) and Alcian blue-positive material (pale gray) are present in the altered intima. Fixed in F. S. A. Pentachrome I stain; × 730.

In the normal valve (Fig. 1) stained with Pentachrome I the elastic membranes and fibers of the auricularis layer stand out in dark purple to black, with pale blue ground substance between them. The fibrosa is composed of yellow collagen, black elastic fibers, and scanty blue ground substance. In the spongiosa there are abundant blue-staining ground substance and scattered yellow collagen fibers. The myocardium stains red and the nuclei are black. The interstitial tissue of the myocardium is blue; only the coarse collagen fibers are yellow.

In experimental hypersensitivity increased or swollen interstitial connective tissue of the

Fig. 6.—Pericardium, acute disseminated lupus. Deposition of fibrinoid into loose edematous tissue. The Alcian blue-positive mucinous edema is most marked around vessels. Fixed in 10% formalin, postfixed in F. S. A. Pentachrome I stain; × 261.

myocardium is well demonstrated (Fig. 2). This tissue stains blue and is partly homogeneous and partly fibrillar. In Figure 3 an acellular sclerosis of the myocardium is seen in a hypersensitized rabbit. Most of the section represents areas of muscle loss with replacement by Alcian blue-positive material. The latter is seen to be partially converted into red-staining collagenous tissue. Myocardial fibers are yellow and nuclei, black.

In the aorta the mucopolysaccharides and their relation to the elastic fibers and membranes have been demonstrated repeatedly quite well with metachromatic dyes.† However, staining with toluidine blue and other metachromatic dyes does not permit counterstaining. Moreover, the metachromasia is lost, at least in part, by dehydration. In the aorta stained with Pentachrome I, the smallest traces of Alcian blue-positive material, presumably acid mucopolysaccharides, are detectable (Fig. 4). In addition, the smooth muscle and cytoplasm of fibroblasts are well shown in red, and nuclei in intense black, the collagen in yellow. Increase of acid mucopolysaccharides, imbibition of plasma, and aging of connective tissue ground substance can be well demonstrated in arteriosclerosis.

Fibrinoid in a small artery is demonstrated in red with Pentachrome I (Fig. 5). In this section there is an increase in ground substance and cells. The fibrinoid is deposited between cells and the blue-staining ground substance. The elastica is absent in one area and fragmented in another.

Both fibrinoid and the "mucinous edema" can be visualized in one section, as seen in Figure 6 representing an acute pericarditis in a case of disseminated lupus.

Both stains are useful in clearly defining the normal components of connective tissue and their relation to one another, while at the same time defining quantitative and qualitative alterations in the various components. Where it is important to establish the relation of pathologic changes to normal structure in connective tissue, conventional methods would require at least two serial sections. Since the histotopography may vary slightly even in serial sections, two separate selective stains cannot relate the pathologic changes to the various components of connective tissue as accurately as can be done in a single section.

While the two stains demonstrate equally well the ground substance, nuclei, and elastic tissue, the woodstain scarlet-acid fuchsin is the better cytoplasmic stain. The advantage of saffron as a collagen stain in Pentachrome I is that it does not cover up, like diphenyl fast red, faintly Alcian blue-positive structures such as are seen at times between collagen bundles and fibers. The advantage of diphenyl fast red for collagen is that delicate collagen fibers and certain reticular fibers are well visualized. Fibrinoid is well stained by both methods.

SUMMARY

Two methods are described for the demonstration of all connective tissue elements in a single section. They stain nuclei black, ground substance and certain reticular fibers blue to bluish-green, and elastic tissue dark purple to black. In one variation cytoplasm stains red and collagen yellow; in the other cytoplasm stains yellow and collagen red. Normal and pathologic connective tissues from a number of organs were stained. The relation of the various components is easily seen.

From these studies it is apparent that the methods are useful in the study of the collagen diseases, arteriosclerosis, mesodermal tumors, and other conditions affecting the connective tissue.

Prof. R. H. More gave encouragement and helpful criticism; Dr. S. A. Bencosme gave advice in technical matters; Mrs. A. Movat gave technical assistance, and Mr. P. H. Mott took the photomicrographs.

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Cerebrovascular Accidents

A Study of Two Hundred One Cases

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Although investigations have been carried out covering numerous phases of cerebrovascular disease, the hypotheses concerning the mechanisms responsible for cerebral hemorrhage and infarction remain controversial. Variations in the clinical material from series to series, in the frequency of postmortem examination of the brain, and in the care with which nerve tissue was examined appear to have caused some of the conflicting conclusions. Detailed postmortem examinations in an unselected series of cases thus appear still capable of yielding valuable statistical information concerning cerebrovascular alterations. Such information may then be employed in the evaluation of the prevailing theories of etiology and pathogenesis.

The autopsy service of Ullevaal Hospital, the principal division of the Oslo City Hospitals, offers an excellent opportunity for the postmortem study of cerebrovascular disease. All disciplines of medicine are proportionately represented in the hospital's 2600 beds, which constitute the majority of those available to the inhabitants of Oslo. In addition, affiliation with nursing homes for the aged and chronically ill permits inclusion of material from these institutions. Autopsies are performed in approximately 90% of deaths, thereby allowing the central nervous system to be obtained almost routinely for careful gross and histologic examination. The relative racial uniformity of the population of Oslo, however, allows for some statistical bias in this material.

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MATERIAL AND METHODS

In the seven-month period, Nov. 1, 1951, to June 1, 1952, a total of 610 postmortem examinations, exclusive of stillborn infants, were performed in the Department of Pathology of the Oslo Community Hospitals (Ullevaal Hospital). The brains were removed for examination in 593 cases (97.2%). The clinical record in the remaining 17 cases (2.8%) did not indicate cerebral involvement. An additional 55 cases exhibiting cerebral pathology were examined from sources other than Ullevaal Hospital.

All nervous system tissue was preserved in a 10% formalin solution and sectioned in one to three weeks. Of the 593 cases, 38 were examined only externally and preserved for subsequent anatomic study. In each of these cases the clinical history indicated a cerebral lesion to be unlikely. The remaining brains were sectioned coronally, maintaining intervals of approximately 0.5 cm. as nearly as possible in order to minimize the possibility of neglecting small lesions. For the purpose of clinical pathologic correlation, the study was directed toward the age and sex of the patient; clinical symptomatology; recorded peripheral pressure; nature, location, and extent of the lesion, and the presence of cerebral arteriosclerosis, associated cerebral parenchymal lesions, and cardiac hypertrophy. Arteriosclerosis was considered to be of moderate or severe degree whenever several cerebral vessels exhibited pathologic involvement resulting in diminution by at least one third of the diameter of the vascular lumen of one or more vessels. The heart was considered to be enlarged when it weighed in excess of 350 gm. in female subjects and 400 gm. in male subjects.

RESULTS AND COMMENT

The pathologic alterations leading to diagnosis of a cerebrovascular accident were noted in 145 (23.8%) of the Ullevaal Hospital

series, and similar findings were present in 24 cases from other sources. An additional 15 cases (2.5%) exhibited asymptomatic vascular malformations or unruptured intracranial aneurysms. Thirteen cases (2.1%) from Ullevaal Hospital as well as four cases from other sources were clinically diagnosed as cerebrovascular accidents, but postmortem examination failed to reveal any parenchymal alteration. The frequency of the various lesions is summarized in Table 1, and the age distribution is noted in Table 2.

TABLE 1.—*Cerebrovascular Disease*

	Ullevaal Hospital, 610 Consecutive Autopsies		Other Sources,	
	No. of Cases	Per Cent	No. of Cases	Total
Cerebral arteriosclerosis				
Slight	130	21.3	4	134
Moderate or severe	185	30.3	29	214
Recent encephalomalacia	32	5.2	10	42
Old encephalomalacia	87	14.3	19	106
Parenchymal hemorrhage	37	6.1	2	39
Intraventricular hemorrhage, source undetermined	1	0.2	0	1
Ruptured aneurysm	13	2.1	0	13
Unruptured aneurysm	9	1.5	0	9
Subarachnoid hemorrhage, source undetermined	3	0.5	0	3
Venous thrombosis	1	0.15	0	1
Telangiectases	6	1.0	0	6
Symptoms of CVA without pathologic change	13	2.1	4	17

TABLE 2.—*Age Distribution of Cerebrovascular Accidents, Ullevaal Hospital*

		Age, Yr.		
	No. of Cases	Below 60, %	60-79, %	80-100, %
Recent encephalomalacia	32	9.4	84.3	6.3
Old encephalomalacia	87	12.6	64.4	23.0
Recent hemorrhage	37	35.1	59.5	5.4

The frequency of cerebrovascular accidents in the present investigation is greater than that noted in earlier studies. Zimmerman [1] reported an incidence as low as 5% in 4240 consecutive autopsies, while Courville [2] noted approximately 11% in 40,000 autopsies. Approximately 15% of Adams and Cohen's [3] series of 2670 autopsies and 16.7% of the 1000 postmortem examinations in Dozzi's [4] report exhibited the pathologic characteristic of cerebrovascular accidents.

These discrepancies may be attributed to factors exceedingly difficult to control. Among the greatest variables in any such study is the population from which the material is drawn. Although each report emanated from a "general hospital," some selectivity necessarily occurred depending on the location of the hospital, the economic status of the patients, and particularly the emphasis or lack of emphasis on neurological or neurosurgical services. Additional factors of importance include the frequency of the postmortem examination, prevalence of brain examinations in routine autopsies, and the care with which the central nervous system is examined. It is apparent that studies in which only a small proportion of brains were examined cannot be adequately compared with those in which the central nervous system was studied in a large percentage of autopsies. The listed percentage of postmortem examinations of the brain varied from 13.8 to approximately 60.* Comparison is still more difficult in other investigations † in which no indication is given as to the autopsy percentage or the frequency with which the brain is examined.

The present investigation was intended to obviate many of these difficulties by the nearly routine pathologic examination of the nervous system in all deaths occurring in an institution which exhibits little selectivity in the patient population. The higher incidence of cerebrovascular pathology noted appears to stem largely from the discovery of clinically unsuspected lesions. The relatively small number of autopsies in this report (610), the short period of time (seven months), and the relative racial uniformity of the patients result in shortcomings in this study as well.

ENCEPHALOMALACIA

The findings in cases of recent infarction are summarized in Table 3, and those in cases of old encephalomalacia are listed in Table 4. Infarction was the lesion most commonly observed consequent to cerebral vas-

* References 3 and 4.

† References 1 and 2.

cular disease, being noted in 17.9% of autopsies. Contrary to the findings of Aring and Merritt [5] and of Zimmerman,[1] who reported encephalomalacia to be twice as common in males as in females, these lesions were found with approximately equal frequency in each sex.

Cerebral arteriosclerosis was commonly associated with infarction, being of moderate or severe degree in more than 85% of cases. Hypertension accompanied encephalomalacia almost as frequently as did arteriosclerosis. Fifty-seven per cent of cases exhibited a recorded blood pressure in excess of 170/100. In an additional 30% of cases a left ventricular type of cardiac hypertrophy, in the absence of valvular disease, indicated the likelihood of hypertension, although blood pressure elevations were not recorded during the hospital stay. Other associated conditions which may have been related to the production of the cerebral softening included two instances of post-traumatic increase of intracranial pressure, two of embolic phenomena, two of severe cardiac failure, one of neoplastic pressure on the basal branches of the middle cerebral artery, one of postoperative shock, and one case of eclampsia.

TABLE 3.—*Recent Encephalomalacia, Forty-Two Cases*

	No. of Cases	Per Cent
Single recent lesions	35	83.3
Multiple recent lesions	7	16.7
Accompanying old lesions	12	27.6
Arteriosclerosis, moderate or severe	36	85.7
Myocardial infarction		
Recent	7	16.7
Old	3	7.1
Blood pressure exceeding 170/100	24	57.1
Cardiac hypertrophy in absence of recorded blood pressure elevation	13	31.0

TABLE 4.—*Old Encephalomalacia, 106 Cases*

	No. of Cases	Per Cent
Single lesions	43	40.6
Multiple lesions	59	55.7
Lesions evident only microscopically	4	3.7
Cerebral arteriosclerosis, moderate or severe	93	87.7
Accompanied by recent CVA	28	26.4
Myocardial infarction		
Recent	21	19.8
Old	19	17.9
Myocardial fibrosis	23	21.7
Blood pressure in excess of 170/100	61	57.5
Cardiac hypertrophy in absence of recorded blood pressure elevation	32	30.2

In more than 80% of cases the initial infarctive episodes were not fatal, indicating the good prognosis for survival. The multiplicity of lesions in over 50% of the cases demonstrates that surviving patients are exceedingly prone to experience one or more subsequent attacks. The older lesions were most commonly located in the basal ganglia, followed by the cerebral cortex and thalamus. This distribution resulted in gray matter lesions outnumbering those in white matter in a ratio of more than four to one.

When postmortem examination revealed a recent encephalomalacia, clinical symptomatology indicative of a cerebrovascular accident had usually been evident prior to death, being noted in 39 of the 42 cases. However, death was not always a consequence of the cerebral involvement. Death resulted directly from the cerebral insult in 29 instances (68%). In an additional six cases (14%) the clinical and pathologic evidence did not allow differentiation between cerebral or myocardial infarction as the cause of death. In seven cases (17%) death resulted from extracerebral conditions, the cerebral accident serving only as a contributing factor.

In contrast to recent encephalomalacia, old cerebral infarcts were commonly unrecognized clinically. In 40% of cases the clinical record gave no suggestion of the infarctive episode. In some instances the lack of recorded symptomatology was a result of location of the lesions in a clinically "silent" area. However, in many other cases careful questioning may have revealed previous transient episodes of faintness, giddiness, speech disturbances, confusion, blindness, or localized weakness which could have led to the correct diagnosis.‡

Most cerebral malacic lesions are too small to permit identification of the responsible

‡ References 6 and 7.

vessel and search for the site of vascular occlusion. However, arterial thrombi were often undemonstrable even in larger vessels supplying an infarcted area. Hicks and Warren [8] emphasized this difficulty in being unable to demonstrate such a thrombus in 60% of a series of 100 cases.

The frequent failure to find an organic vascular occlusion has led many investigators to incriminate vascular spasm as the cause of such infarctions. Support of this hypothesis has been justified by the following observations: 1. Spasm has been produced in experimental animals by mechanically stroking or electrically stimulating the cerebral vessels, as well as by the intravascular injection of ground pumice emboli.[9] 2. Spasm of the pial vessels has been visualized at surgery during a convulsive seizure.[10] 3. Anesthetic block of the cervical sympathetic ganglia has been reported to produce clinical improvement in cases of cerebral infarction. 4. Spasm of the larger vessels accompanying ruptured aneurysms of the circle of Willis has been diagnosed angiographically.[11]

The following evidence suggests that spasm does not play a significant role in the production of encephalomalacia: 1. Experimental production of spasm necessitates measures more drastic than those usually encountered in human vasculature, even during infarction. In addition, such procedures have too infrequently led to encephalomalacia.[9] 2. Despite surgical visualization of constricted pial vessels during seizures, the cerebral blood flow has been demonstrated to be increased under these conditions.[12] 3. Studies indicating the efficacy of stellate ganglion block in cerebral infarction have been too poorly controlled to be accepted as conclusive evidence. Physiologic studies have demonstrated, contrariwise, that no increase in cerebral blood follows this procedure in either normal persons or patients with acute cerebral infarcts.[13] 4. Similar investigations have demonstrated the cerebral arteries to be capable of some contraction in response to appropriate stimuli such as carbon dioxide lack and oxygen excess. Conversely, considerable vasodilation results from carbon dioxide excess and oxygen lack such as prevails during any infarctive episode.[14] 5. Cerebral malacic lesions most commonly occur in areas supplied by the smaller cerebral arteries. These vessels are incapable of any significant degree of spasm, since their media contain only minimal amounts of smooth muscle.[15] 6. The extremely high incidence of cerebral arteriosclerosis accompanying encephalomalacia makes even the larger vessels incapable of spasm in the majority of cases.

Previous physiologic studies of cerebral circulation together with the results of the present investigation suggest some other mechanism to be responsible for the production of encephalomalacia in the absence of any pathologically demonstrable vascular occlusion. Infarction results whenever the blood supply is inadequate for cerebral metabolic requirements. The supply is regulated by the caliber of the vascular lumina as well as the cardiac ability to deliver blood. The patency of the cerebral vessels is influenced by physiologic factors as well as the anatomic changes produced by atherosclerosis. The physiologic factors operate to maintain a relatively constant cerebral blood flow under normal conditions in normotensive persons.[12] These factors produce their most significant changes in the direction of vasodilation, while constriction may occur only to a lesser degree. In hypertension, which commonly accompanies encephalomalacia, the "resistance" to cerebral blood flow is elevated to prevent the cerebral circulation from exceeding normal levels [16]; conversely, lowering of the peripheral blood pressure results in some degree of vascular dilatation, diminishing the cerebrovascular resistance, and allowing a normal rate of blood flow. The ability rapidly to decrease resistance is altered in hypertensive persons; consequently, vascular dilatation may not adequately and rapidly compensate for blood pressure drops. The resultant diminution in cerebral blood flow produces a serious danger of cerebral ischemia.[16]

Cardiac damage of some degree almost uniformly accompanies encephalomalacia.[17] Such

damaged hearts are susceptible to episodes of decompensation, which, in turn, produce a decrease in the cerebral blood flow. A mean diminution of 39% in the cerebral blood flow has been demonstrated in such patients with cardiac failure.[18]

The physiologic deficiency produced by cardiac failure and blood pressure drops in hypertensive persons may be locally accentuated by narrowing of the vascular lumina by atherosclerotic plaques. In addition, the likelihood and site of infarct formation may be influenced by cerebral blood demands as well as deficiencies in supply. The gray matter oxygen demand greatly exceeds that of white matter and is further accentuated by functional activity.[12] The increased demand of gray matter may thus account for the marked predominance of gray matter infarctive lesions over those in white matter.

This evidence suggests that a number of factors operate to diminish the cerebral blood supply of hypertensive persons. These periodic episodes of deficiency in supply are accentuated by the high oxygen demand of gray matter. Thus infraction may occur, particularly in cerebral gray matter, whenever the demand exceeds the supply, despite the absence of a demonstrable occlusion of the responsible vessel.

Hemorrhagic Infarction

Eleven cases of acute infarction exhibited the pathologic characteristics of a hemorrhagic infarct. In an additional five cases small portions of the lesions were hemorrhagic. Such infarcts have been postulated to result from alterations in arterial flow followed by a disturbance of venous return,[10] as well as from preexisting vasodilation and stasis with subsequent arterial occlusion.[20] Partial arterial blockage has also been implicated, as have embolic phenomena with transient vascular occlusion.[21] Experimentally, hemorrhagic infarcts have been produced by temporary or permanent arterial occlusion.§

The almost exclusive distribution of hemorrhagic infarcts in areas demarcated by arterial supply, in this investigation, is consistent with the predominantly arterial origin of these lesions. In the majority of such cases a venous component may have accompanied the arterial alteration. Arterial occlusion without interference of venous return was indicated in a single case, in which the basal branches of the middle cerebral artery, the so-called lenticulostriate branches, appeared to be compressed by a small suprasellar meningioma (Fig. 1). A unilateral hemorrhagic infarction was thus produced in the globus pallidus and putamen. No evidence of increased intracranial pressure was noted, while the anatomic location of the Galenical veins draining this area excluded them from pressure by the neoplasm.

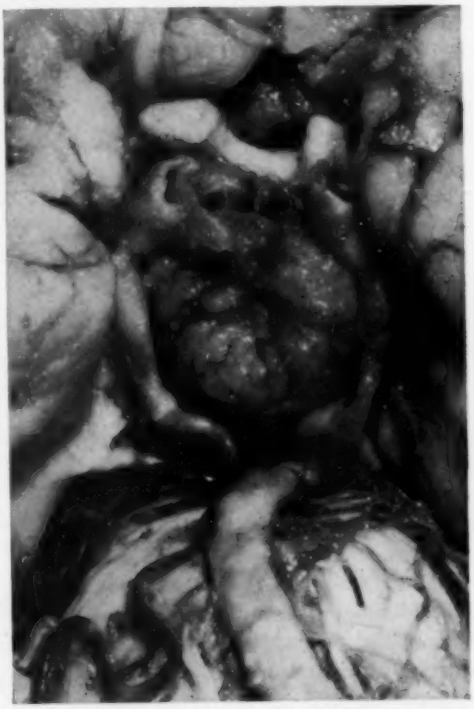

Fig. 1.—Suprasellar meningioma compressing the basal branches of the middle cerebral artery. This lesion resulted in unilateral hemorrhagic infarction of the globus pallidus and putamen.

No evidence of hemorrhagic infarction of embolic etiology was noted in this series. Only two instances of acute anemic in-

§ References 22 and 23.

farction resulted unquestionably from emboli. While this investigation does not exclude emboli as a cause of hemorrhagic infarction, it suggests that other forms of arterial blockage are commoner.

The frequency of hemorrhagic phenomena in cerebral infarction carries therapeutic implications. No factor was discerned enabling clinical differentiation between hemorrhagic and anemic infarcts. Administration of bishydroxycoumarin (Dicumarol) or other anticoagulants always carries the strong possibility of converting a hemorrhagic infarct into a frank hemorrhage. Anticoagulants are thus strongly contraindicated in most cerebrovascular accidents, even when it is possible to differentiate infarction from hemorrhage.

TABLE 5.—*Cerebral Hemorrhage, Thirty-Nine Cases*

	No. of Cases	Per Cent
Single hemorrhage	32	82.1
Multiple hemorrhages	7	17.9
Secondary pontine hemorrhage	6	15.4
Arteriosclerosis, moderate or severe	28	71.8
Myocardial infarction		
Recent	5	12.8
Old	2	5.1
Blood pressure in excess of 170/100	25	64.1
Cardiac hypertrophy in the absence of recorded blood pressure elevation	13	33.3

HEMORRHAGE

Central nervous system hemorrhage was observed only slightly more frequently in postmortem material than was acute encephalomalacia (Table 5). As noted by Aring and Merritt [5] and Zimmerman,[1] these lesions occurred with about equal frequency in either sex. Unlike infarctive episodes, cerebral hemorrhage most commonly terminated fatally. Occasionally small cystic areas were observed in which the density of hemosiderin deposition indicated a previous small hemorrhage; however, none of the large lesions exhibited the characteristics of an earlier hemorrhage. Increased intracranial pressure appeared to be an important factor in this difference in survival. The pressure was sufficiently elevated in six cases of cerebral hemorrhage to produce secondary pontine hemorrhages, while this finding was noted in only a single case of encephalomalacia.

Other marked differences were noted between cerebral hemorrhage and encephalomalacia. As recorded in other studies,[5] the age distribution was lower in cases of fatal hemorrhage, over one third of the patients being below 60 (Table 2). This group also exhibited severer hypertension, while arteriosclerosis, though common, was a less frequent occurrence in hemorrhage than in encephalomalacia (Table 5). The younger age of patients dying of cerebral hemorrhage may account in some degree for the lower incidence of vascular sclerosis. A previous episode of encephalomalacia was frequent, being noted pathologically or suggested by the medical history in almost half the cases. Other findings associated with cerebral hemorrhage included single instances each of acute purulent meningitis, polycythemia, and acute leukemia. The clinical history was uniformly characteristic of a cerebral hemorrhage.

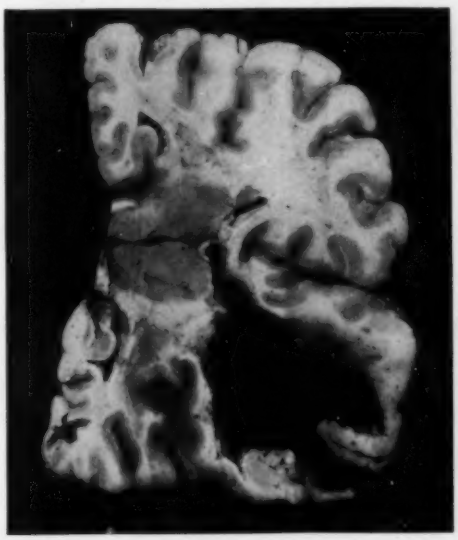

Fig. 2.—Massive cerebral hemorrhage involving the cerebral white matter. The basal nuclei and thalamus are uninvolved. Rupture occurred into the subarachnoid space.

The bleeding most commonly was massive, often obscuring the site of origin. The most characteristic lesion appeared to be located

predominantly in the cerebral white matter, although the basal ganglia, internal capsule, or thalamus as well were frequently involved (Fig. 2). This pattern was observed in 51% of the 39 cases. In an additional five cases the hemorrhage principally involved the cerebellar white matter. In seven cases the hemorrhages were small and multiple and chiefly involved the cerebral gray matter. The incidence of primary pontine hemorrhage in seven cases exceeds the incidence noted by other investigators,[24] but this may be a reflection of the small number of hemorrhages in this study.

The exact pathogenesis of cerebral hemorrhage is still a source of hypothesis. On occasion, a rupture site can be located in an arteriosclerotic vessel. However, the vascular rupture is rarely found, and cerebral arteriosclerosis may be insignificant. Among the theories attempting to elucidate this occurrence is that proposed by Rouchoux and championed by Globus.[25] An initial softening was postulated, leading to loss of the structural support of certain vessels. The unsupported vessels were then assumed to be more prone to rupture.

The finding of hemorrhage in areas of previous softening in five cases in this series would seem to add strength to this hypothesis. However, such a theory still fails to account for the predominance of lesions in gray matter, while hemorrhage predominates in white matter. In addition, hemorrhage was only uncommonly observed in the occipital cortex, although this area is a frequent site of softening. The younger age distribution in cases of hemorrhage also appears to be against this hypothesis.

Aneurysms

The incidence of intracranial aneurysms in this series exceeded the previously recorded frequencies of 0.5% to 1.5%.[26] Of the Ullevaal Hospital autopsies, 2% exhibited ruptured intracranial aneurysms, while an additional 1.5% exhibited unruptured aneurysms (Table 1). However, the 22 cases studied are too few to endow statistical significance to this difference. The finding of a predominance of intracranial aneurysms in female patients, in a ratio of 13 to 9, is consistent with other reports.[27]

Recent investigations indicate the middle cerebral artery and the anterior cerebral-anterior communicating artery junction to be most commonly involved by intracranial aneurysms.‖ This distribution holds true in the present investigation, with 70.9% of aneurysms in these two locations (Table 6). The single commonest location was the first bifurcation of the middle cerebral artery. The surgical approach to such a lesion involves considerable risk of hemiplegia. When the involved artery is in the dominant hemisphere, the additional likelihood of aphasia generally contraindicates surgical procedures.

Table 6.—*Location of Intracranial Aneurysms*

	No. of Cases	Per Cent
First bifurcation of middle cerebral	12	38.7
Anterior communicating-anterior cerebral	10	32.2
Second bifurcation of middle cerebral	3	9.7
Middle cerebral-anterior cerebral	2	6.5
Internal carotid	1	3.2
Basilar	1	3.2
Posterior cerebral, cortical branch	1	3.2
Anterior cerebral, distal to anterior communicating	1	3.2
	31	

In more than half the cases of aneurysm of the anterior communicating artery region, the caliber of one of the anterior cerebral arteries proximal to its junction with the anterior communicating artery (Fig. 3) was structurally insufficient, necessitating the derivation of blood supply in its distribution from the contralateral anterior cerebral artery. In another report,[32] this abnormality in circulation was noted to be rare in routine autopsies, although commonly associated with aneurysms of this region. Ligation of the remaining patent anterior cerebral artery or any interference with blood flow through the anterior communicating artery carries the danger of unilateral or bilateral impairment of anterior cerebral circulation.

The risk of aphasia and hemiplegia in aneurysms of the middle cerebral artery sup-

‖ References 26 and 28 through 31.

plying the dominant hemisphere and the danger of compromising the circulation in cases of aneurysm of the anterior communicating artery region indicate that almost one third of intracranial aneurysms are in situations too hazardous to be treated by direct surgical approach. Therefore, intracranial surgery for ruptured aneurysms will be most effective in regions where these dangers are not encountered.

Fig. 3.—Aneurysm of the right anterior cerebral artery. There is approximate three-fold disparity in size between the two anterior cerebral arteries.

The intracranial hemorrhage was the cause of death in each of the 13 cases of fatal aneurysmal rupture. As noted in previous investigations,[26] extravasation of blood into the cerebral parenchyma commonly accompanied cases of fatal ruptured aneurysms. This suggests that such bleeding may play a significant role in producing death. This type of cerebrovascular accident commonly produces recognizable clinical involvement. In 12 of the 13 cases in this series the cerebrovascular accident was evident in the clinical history. No history was available in the 13th case, since the patient was found dead.

The occurrence of aneurysms in several elderly patients (Table 7) demonstrates that advanced age alone is insufficient to rule out aneurysms as a cause of cerebrovascular accidents. Angiography to substantiate the diagnosis is generally contraindicated in these patients by the frequency of arteriosclerosis and the consequent danger of precipitating a cerebral infarction.

Although the etiological factors in the formation of berry aneurysms have not been completely elucidated, two theories are most generally accepted. The first was popularized by Forbus,[33] who noted that aneurysms arose at the bifurcation of vessels in which medial

TABLE 7.—*Age Incidence of Intracranial Aneurysms*

	Age, Yr.			
	40-49	50-69	70-89	90
Ruptured aneurysms	2	8	2	1
Unruptured aneurysms	..	3	6	..

defects were present. This defect has not been considered to be the sole factor, since such alterations occur in uninvolved cerebral vessels as well as in extracranial locations where aneurysms are rare. Bremer [34] believed that medial defects constituted one factor but advanced a second theory suggesting a failure of complete involution of a primitive embryonic vascular plexus. Other hypotheses have implicated defects in the vascular elastica [35] and arteriosclerosis.[36]

The present investigation does not delineate the mechanism most commonly responsible for the production of aneurysms. The presence of a single aneurysm on the anterior cerebral artery away from a bifurcation, but in the region of the primitive vascular plexus (Fig. 3), suggests that this aneurysm represents an incompletely involuting vascular remnant. Arteriosclerosis was present in only 7 of the 13 cases of ruptured aneurysm (Table 8), indicating that vascular sclerosis is not an essential etiological factor

in the formation of berry aneurysms. Hypertension was present in 10 of the 13 instances of ruptured intracranial aneurysm, but it was noted in less than half the cases of unruptured aneurysm (Table 8). This suggests that hypertension is unrelated to aneurysmal formation but a possible factor in determining which aneurysms rupture.

Vascular Malformations

Vascular malformations are an infrequent cause of cerebrovascular accidents. The only lesions of this nature encountered in the present investigation were six asymptomatic cases of telangiectasis. These abnormal capillary dilatations rarely result in cerebral symptoms; however, a possible role in the causation of cerebral hemorrhage has been indicated by Margolis and his associates.[37]

Table 8.—*Intracranial Aneurysms, Twenty-Two Cases*

	No. of Cases	Per Cent
Ruptured	13	59.1
Unruptured	9	40.9
Single	14	63.6
Multiple	8	36.4
Previous subarachnoid hemorrhage	2	9.0
Cerebral arteriosclerosis, moderate or severe	12	54.5
Acute myocardial infarction	2	9.0
Hypertension		
Ruptured aneurysms	10	76.9
Unruptured aneurysms	4	44.4

Venous Thromboses

The infrequency with which thrombi of the cerebral veins produce a cerebrovascular accident was indicated by the presence of only one such case in the present series. This condition has reportedly been associated with the puerperium, arthritis, infections, surgical procedures, infectious diseases, and blood dyscrasias. Smith [38] also suggested the possibility of primary thrombosis of the cerebral veins being a manifestation of thromboangiitis obliterans. The case in the present series was seen in a mentally deteriorated 89-year-old man with hypertension. The histological examination of the involved cerebral vein revealed infiltration of the vessel wall with mononuclear cells. This suggests that inflammation of the vascular wall was the initial alteration which resulted in thrombosis.

Subarachnoid and Intraventricular Hemorrhage of Undetermined Origin

Despite careful search for possible sources of bleeding, three cases of subarachnoid bleeding were observed in which no ruptured aneurysm could be demonstrated. In one case clotted blood in the Sylvian fissure was highly suggestive of an aneurysm of the middle cerebral artery. In the remaining two cases no local accumulation of blood was noted, although it seemed likely that the bleeding was consequent to an unlocated ruptured aneurysm or to a small vascular malformation obscured in a sulcus. Subarachnoid bleeding consequent to necrosis of a cerebellar or vertebral artery has been described by Kernohan and Woltman [39] and may be considered as a possible source of bleeding in cases such as those observed in this series.

Intraventricular bleeding unaccompanied by subarachnoid or parenchymal hemorrhage was noted in a single case in which no source of the blood could be found. The rupture of a subependymal vein or a rare intraventricular aneurysm of the anterior choroidal artery are among the possible sources of this hemorrhage.

Symptoms of Cerebrovascular Accident Unaccompanied by Pathologic Lesions

The vagueness of the symptoms did not allow adequate substantiation in 11 of the 17 patients for whom a diagnosis of cerebrovascular accident was recorded. Further evidence against the occurrence of a cerebrovascular accident in most of these cases was the association of myocardial hypertrophy or hypertension in only five cases, although one or both of these findings was almost uniform in actual cerebrovascular accidents. Six cases still remained in which the history and accompanying cardiovascular findings made it reasonable to assume that a cerebrovascular insult occurred. In two, a duration of less than 24 hours was too brief to allow the development of visible pathologic alterations.

The time interval and clinical evidence in the remaining four cases indicated that a sizable infarction could reasonably have been expected. It must then be assumed that occasional alterations in function may occur without structural changes demonstrable by the techniques now in routine use.

Association of Cerebrovascular Accidents with Myocardial Infarction

Myocardial infarction is a frequent concomitant of cerebrovascular accidents. Dozzi [40] noted that 11.2% of 107 cases of cerebrovascular accidents were accompanied by myocardial infarction. In 29% of 41 cases of coronary thrombosis he also noted an accompanying cerebrovascular lesion. He subsequently reported 12.1% of 66 hemiplegia patients as presenting electrocardiographic evidence of myocardial infarction.[41] Race and Lisa,[42] in 100 consecutive autopsies of either myocardial infarction or an acute cerebrovascular accident, noted 15 instances in which these two lesions were combined. Bean and associates [43] reported six cases of hemiplegia following acute myocardial infarction. In the present investigation, acute myocardial infarction accompanied cerebral hemorrhage in 13%, recent encephalomalacia in 16.7%, and ruptured aneurysm in 15.4% of cases.

The myocardial and cerebral episodes may both be manifestations of a hypertensive state; thus, the same group of patients tends to exhibit one or both lesions. This is well reflected in the 37.7% of the 106 cases of old encephalomalacia demonstrating old or recent myocardial infarction. When acute, these conditions tend to occur simultaneously, since the shock resultant from either variety of infarction may sufficiently diminish the already incompetent circulation to produce infarction of the other organ. The abnormal tendency for rapid blood clotting observed in acute myocardial infarction has been suggested as a possible factor in this combination of diseases.[43]

Summary and Conclusions

Of 610 consecutive autopsies, 25.7% exhibited pathologic lesions characteristic of a cerebrovascular accident. The following conclusions were reached in the study of these cases:

Infarction is the commonest parenchymal lesion resulting from disease of the cerebral vessels. These lesions are usually nonfatal and are most frequent in the cerebral gray matter.

Cerebral infarction was clinically unrecognized in nearly 40% of cases exhibiting nonfatal lesions. In contrast, fatal cerebrovascular accidents are almost uniformly clinically evident. On rare occasion they may be obscured by other diseases.

Hypertension and myocardial hypertrophy are extremely frequent concomitants of cerebral infarction or hemorrhage.

Patients exhibiting cerebral hemorrhage tend to die at an earlier age and exhibit severer hypertension than do those with infarction.

Encephalomalacia in the absence of demonstrable vascular occlusion is considered to result from a combination of factors reducing the blood supply and increasing the oxygen demand. Factors reducing the blood supply include vascular sclerosis, cardiac impairment, and the limited ability of hypertensive persons to maintain cerebral blood flow in the face of transient drops in the peripheral blood pressure. Variations in oxygen requirements temporarily increase demand and appear responsible for the predominance of infarctive lesions in cerebral gray matter.

Hemorrhagic infarcts cannot be clinically differentiated from anemic infarcts. Anticoagulants are generally contraindicated because of danger of converting a hemorrhagic infarct into frank hemorrhage.

Intracranial aneurysms are most commonly located on the middle cerebral artery or in the region of the anterior communicating artery. The danger of aphasia or of compromising the anterior cerebral circulation contraindicates direct surgical approaches in many of these cases.

In rare instances clinical evidence of cerebrovascular accident may appear unaccompanied by a demonstrable morphologic change.

Acute myocardial infarction accompanied recent cerebrovascular accidents in approximately 15% of cases.

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Chemical Nature of the Storage Substance in Gargoylism

Hurler-Pfaundler's Disease

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The rare familial disorder commonly designated as gargoylism is presently recognized as constituting a distinct clinical and pathological entity, but its exact place in the category of "storage diseases" is not satisfactorily defined. The disease is characterized by disproportionate dwarfism, skeletal deformities, hepatosplenomegaly, tendency toward formation of umbilical and inguinal hernias, cloudiness of the corneas, and mental retardation. Microscopically the remarkable feature is the storage of an unknown material, in a diffuse and variable manner, in liver cells, endothelial lining cells of the spleen and lung, the connective tissue of cardiac valves, cartilaginous tissue, Bowman's layer of the cornea, and neuronal bodies of the central nervous system. Profound alterations in the appearance and staining characteristics of connective tissue in general are also observed.* A high percentage of cases is noticeable for the peculiar metachromatic stippling of leucocytes (Alder bodies) that is present.[8] An enlargement of the sella turcica, probably due to osseous anomalies rather than expansion of the pituitary gland, has also been repeatedly observed.† After the first report of Hurler [6] and its evaluation as a disorder of pronounced individuality by Pfaundler,‡ the disease has been variously referred to as Hurler-Pfaundler's syndrome, dysostosis multiplex,[9] lipochondrodystrophy,[9] and gargoylism,[10] depending on the preferences of individual workers in stressing the unknown nature of the nosological entity, the profound alterations in the skeletal system it presents, the generalized visceral involvement with storage of "lipid-like" material, or the grotesque appearance that the afflicted patient manifests. Recent studies by Reilly and co-workers § and Lindsay and associates [1] have increased considerably our knowledge of the generalized involvement of organs, the histological characteristics of involved tissues, and the histochemical reactions of the storage substance that is deposited in various organs. Lack of information on the chemical nature of the storage substance has, however, made it difficult to formulate a pathogenesis or to indicate where the genetically induced metabolic error may lie. Although some patients do have deposits that take the conventional fat stains ‖ and the neuronal involvement presents histological features indistinguishable from those found in Tay-Sachs' disease,¶ careful analytical work # has shown that there is no significant change in the lipid constituents of involved

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* References 1 and 2.

† References 4 through 7.

‡ Pfaundler, cited by Reilly and Lindsay [11] and Thannhauser.[17]

§ References 3 and 11.

‖ References 1, 4, and 12 through 14.

¶ References 4, 15, and 16.

References 2, 17, and 18.

organs as determined by the usual analytical methods and, as such, gargoylism does not merit the designation of "the fifth lipoidosis."* Histochemical studies have indicated that the material can be stained with periodic acid-Schiff's reagent (PAS), and that it sometimes gives a positive reaction with Best's carmine stain. Erroneous conclusions drawn from these staining characteristics have led some workers [1] to postulate that gargoylism represents a form of glycogen-storage disease akin to von Gierke's disease, especially in view of the cardiomegaly, with carbohydrate-like material that is stored in the tissues between the striated muscle fibers and valvular connective tissue, and the enlargement of the liver. Most pathological studies have shown the finding of large vacuolated cells in the liver † in formalin-fixed or alcohol-fixed tissue, and it is unavoidable to conclude that the storage material was washed out during such fixation. The dioxane-dinitrophenol fixative of Lindsay and co-workers [1] serves to avoid this artifact, and histochemical studies on the storage material have thus become practicable. From such studies these workers arrived at the very astute conclusion that the storage material might be glycoprotein in nature. The manifestly polysaccharide character exhibited by its staining with periodic acid-Schiff's reagent and Best's carmine stain also led these workers to speculate on the relationship of gargoylism to von Gierke's disease, although it appeared quite clear that the material stored was not glycogen. The most significant contribution to the subject is probably that of Brante, who recently reported [19] histochemical studies that led him to believe that the storage material consisted of at least 50% gangliosides, the rest representing an unknown substance probably lipid in nature. In a later work, however, the same author reported [20] on the isolation of a material polysaccharide in nature, having 3.9% sulfur, 27% hexosamine, and 26% glucuronic acid in its composition. There were no fatty acids present in the hydrolysate. The material was reported to be present in an amount corresponding to 10% of the dry weight of the liver. From these compositional characteristics Brante concluded that gargoylism was characterized by the storage of a mucopolysaccharide and that the disease should hence be classified as a mucopolysaccharidosis. It is unfortunate that detailed data on the isolation are not given by Brante and that the material is inadequately characterized to justify such a conclusion in conflict with his earlier careful work. The investigations reported here were started in 1951 and, by the very nature of studies dependent on tissue obtained from rare cases, have only recently been completed, being based on material obtained from four patients. The storage material was isolated from the surgically removed spleens of two patients and the liver and spleen of two other patients on whom postmortem examinations were done. The results of our isolation procedures show the storage material in gargoylism to consist of two major fractions differing in chemical composition and physical properties, but neither of them being a mucopolysaccharide in the generally accepted sense. One of these fractions is a glycolipid of the ganglioside type that forms a complex with one or more smaller peptides, while the other is a complex polysaccharide containing only a small amount of hexosamine. The latter also contains sulfur that is released as sulfate on hydrolysis and, as such, may be related to the preparation reported by Brante, and possibly to the "keratosulfate" recently isolated by Meyer and co-workers [21] from the bovine cornea.

MATERIAL ‡

The material used in this study had the following sources:

CASE 1.—A 9-year-old boy with mental retardation, hepatosplenomegaly, umbilical hernia, slight

* References 12 and 13.

† References 2, 14, and 17.

‡ Mr. William Pfeffer supplied the material from three of the cases cited, and Dr. Donald Jolly made available the spleen and liver of one patient who died at the Wrentham State School, Mass.

Detailed clinicopathological studies will be reported separately.

cloudiness of the corneas, and with the classical skeletal and skull changes of gargoylism. The spleen was removed surgically and frozen immediately in a dry-ice chest.

CASE 2.—A 20-year-old woman who died of intercurrent infection at the Wrentham State School. The liver and spleen were removed at autopsy about eight hours after death and frozen solid in a dry-ice chest until processed.

CASE 3.—An 11-year-old girl admitted to the Boston Children's Hospital with the characteristic findings of gargoylism. A sibling had previously died with the same condition. The liver and spleen were removed about eight hours after death and frozen solid.

CASE 4.—A 5-year-old boy presenting the classical picture of gargoylism, with corneal cloudiness, umbilical hernia, marked hepatosplenomegaly, and mental retardation, etc. The spleen was removed surgically and stored in a dry-ice chest until processed.

ISOLATION OF THE STORAGE MATERIAL

In attempting the isolation of the storage material, observations made by previous workers on the solubility and histochemical properties of the substance were used as guides for the choice of suitable solvents. Brante had observed that microscopic sections of the involved tissue placed in formalin appeared to lose some material to the ambient aqueous solvent, and that the addition of thionine blue caused the appearance of a reddish metachromatic sediment in the ambient fluid around the tissue slice.[19] Also, the material appeared to dissolve in aqueous-acetone media.§ Furthermore, it was obviously not a component of the lipid fractions obtained from tissue when ether and alcohol-chloroform ‖ were used as the extracting solvents.

Basing pilot experiments on these observations, two procedures for the isolation of the storage material were developed. In both cases the storage material was recovered in the form of two distinct fractions.

METHOD 1.—*Aqueous-Acetone Procedure.*—The method is based on the solubility of the complex storage material in aqueous acetone, to the exclusion of most of the tissue proteins, and the subsequent removal of neutral fat, fatty acids, and cholesterol from the aqueous-acetone phase by exhaustive partitions against chloroform, in which solvent the storage-material complex is quite insoluble. The tissue (spleen or liver) was homogenized with acetone (7 ml. per gram of tissue) in a Waring Blendor, and the homogenate thus obtained was gently brought to boiling on a steam-bath and immediately filtered through a Buchner funnel under suction. The residue was discarded. The extract was evaporated to one-fifth of the original volume overnight under a stream of nitrogen. The residue was taken to dryness in a desiccator. The yellow magma thus obtained was extracted with chloroform and the extract discarded (molar nitrogen/phosphorus ratio in extract was 1.21). The material was then dissolved in water and partitioned twice, with two hours' shaking, against chloroform. The aqueous phase was separated and dialyzed against distilled water for 48 hours at 2 C. The dialysate contained some free amino acids, glucose, and other small-molecular tissue components (chromatogram). After dialysis the contents of the bag were lyophilized to yield a yellowish-white, fluffy, hygroscopic powder. The dried powder was dissolved in water (1 gm. per 10 ml.) and centrifuged at 20,000 g for one hour. A gelatinous compact sediment (Fraction P-crude) was separated from a clear, yellowish supernatant (Fraction S crude). Both were lyophilized. Fraction S was soluble in ethanol; Fraction P was not. Both were insoluble in chloroform, chloroform-methanol, petroleum-ether, and benzene. Further purification was carried out as in Method 2.

METHOD 2.—*Formaldehyde Procedure.*—This is based on the fact that the storage material is soluble in formaldehyde, whereas tissue lipids and other macromolecular tissue constituents are not to any significant degree.

The tissue was homogenized with a 20% formaldehyde solution (5 volumes of solvent per gram of tissue) in a Waring Blendor, and the homogenate was heated with thorough stirring on a steam-bath to about 80 C.

§ References 17 and 19.

‖ References 2 and 17.

The homogenate was filtered through filter paper and the residue extracted once more with formaldehyde. The two filtrates were combined and placed in dialysis bags. Dialysis was effected against cold running tap water. The bags were removed after four hours and the contents transferred into new bags, as the osmotic pressure developed within the bags often caused rupture of the casings. Dialysis was continued against tap water for 48 hours until no odor of formaldehyde could be detected and until the dialysate did not produce a pink color with Schiff's reagent. After dialysis the solution was amber-yellow, highly opalescent, and slightly viscous. It was pervaporated to one-third volume in a current of air. The ensuing precipitate was removed by centrifugation and discarded. To the supernatant 1N HCl was added dropwise to pH 3.5. On standing, the initial opalescence of the solution changed to turbidity. At this stage 2 volumes of 95% ethanol was added. The precipitate was allowed to settle out overnight in the cold and recovered by centrifugation (Fraction P crude). The supernatant was pervaporated in a dialysis bag to remove the ethanol, and the clear aqueous solution was lyophilized (Fraction S-crude).

The crude Fraction P was dissolved in a minimal amount of water and reprecipitated with 1½ volumes of ethanol at pH 3.5. It was then redissolved in water and dialyzed 24 hours in the cold. Finally, the aqueous solution was lyophilized to yield a white, fluffy powder.

The crude Fraction S was further purified by extracting the powder with hot chloroform, dissolving the residue in water, and partitioning it several times against equal volumes of chloroform (molar ratio of N/P in chloroform phase was 1.42). The aqueous phase was recovered by siphoning, centrifuged to remove a negligible amount of insoluble material, and the clear pale yellow solution taken to dryness over calcium chloride in an evacuated dessicator. The dried material formed a continuous, transparent, very brittle film which, on being ground in a mortar, was white in appearance. Under polarized light, there was some evidence of polycrystallinity with areas showing high degrees of orientation birefringence. The material was easily soluble in water, methanol, and ethanol, but insoluble in all other organic solvents tested.

Protein-Bound Fraction S.—After exhaustive extraction of the tissue with formaldehyde, the residue was tested for polysaccharide by suspending an aliquot in 1.0 ml. of water to which was added 2.0 ml. of a 1% solution of orcinol in 60% H_2SO_4. The mixture was heated 15 minutes in a boiling water-bath, whereupon a deep red color was observed to develop. It was therefore assumed that a portion of the storage material was firmly bound to protein. This portion was also isolated in the following manner. The dried residue was suspended in M/20 $NaHCO_3$ (100 gm. dry powder per 500 ml.) and was heated in a water-bath at 80 C for one hour with vigorous stirring. The extract was filtered through heavy filter paper, and 3 volumes of ice-cold 10% trichloroacetic acid was added to the slightly turbid, amber-colored solution. The ensuing heavy protein precipitate was removed by filtration. The filtrate was then dialyzed for 36 hours against running tap water to remove the trichloroacetic acid. The clear solution was then concentrated 10-fold by lyophilization. To the concentrate, 3 volumes of ethanol was added, and the ensuing small precipitate was removed by centrifugation. This negligible precipitate proved identical with Fraction P previously described. The ethanol was removed from the solution by pervaporation and the aqueous residue was shaken with chloroform and finally lyophilized. The dried powder consisted of purified Fraction S, being similar in solubility and composition with that obtained from the formaldehyde extract. This fraction was obtained from both of the livers studied, and a negligibly small amount was recovered from a surgically removed spleen (Case 4).

YIELDS

Although the composition of Fractions P and S obtained by the two different proces-

TABLE 1.—*Yields of Fractions P and S Obtained by Two Different Isolation Procedures*

		Gm./100 Gm. Fresh Tissue			
		Method 1		Method 2	
	Fractions	P	S	P	S
Case 1	Spleen	0.42	0.81	0.87	1.12
Case 2	Spleen	0.34	0.82	3.26	1.14
	Liver	0.25	0.51	4.34	1.38
Case 3	Spleen	....	....	0.66	1.40
	Liver	0.28	0.84	0.73	1.43
Case 4	Spleen	0.40	0.74	0.48	1.34

ses of isolation was the same, it is evident (Table 1) that there is a marked difference in the yields. The formaldehyde procedure gave uniformly much higher yields and was therefore given preference in preparing larger amounts of each fraction for studies of chemical composition and physical properties.

CHEMICAL COMPOSITION

For the detection of ninhydrin-positive material and sugar constituents both Fractions P and S were hydrolyzed with 6N HCl in sealed tubes at 110 C for 16 hours, and the acid was removed by taking the hydrolysates repeatedly to dryness *in vacuo*. Hydrolysates were made up to known volumes with water, and appropriate aliquots were used for chromatography. The presence of ninhydrin-reacting substances was detected by paper partition chromatography using phenol-water, butanol-acetic acid-water (65:15:20), butanol-pyridine-water (3:2:1.5), and butanol-acetic acid-ethanol-water (45:10:35:10) in one- and two-dimensional runs. Glucose and galactose were detected according to Novellie's procedures,[22] using β-naphthylamine as the reagent. Quantitative estimation of identified constituents of Fractions P and S was effected by elution of respective spots and nitrogen analysis on the eluates (glucosamine, chondrosamine, sphingosine), determination of the reducing value of eluates (glucose, galactose), or by the use of specific color reactions on the original material. Neuraminic acid was estimated according to Klenk and Langerbeins.[23] Total reducing value was determined by the procedure of Folin and Malmros [24] and expressed as glucose equivalents. The reducing value of eluates of spots corresponding to glucose and galactose was used to compute the glucose: galactose ratio. This was also checked by modifications of the cysteine reaction.[25] Total hexuronic acids were determined by the carbazole reaction,[26] and total hexosamine according to Dische and Borenfreund.[27] The latter agreed well with the results obtained from the nitrogen content of chromatogram eluates of areas corresponding to glucosamine and chondrosamine. Sphingosine was estimated by the nitrogen content of the eluate of a chromatogram developed in butanol-acetic acid-ethanol-water (45:10:35:10:), R_f—0.95. Since the free base as well as sphingosine sulfate ran close to the solvent front in this solvent system, interference from the presence of other nitrogen-containing substances can be neglected. The presence of sphingosine in Fraction S was further established by the isolation of sphingosine sulfate after methanolic-sulfuric acid hydrolysis of the material.[28]

For the estimation of total fatty acids, the material was hydrolyzed for 18 hours at 100 C in sealed alkali-resistant tubes with baryta. On cooling of the hydrolysate, the insoluble barium salts of the fatty acids precipitated out. The precipitate was collected by centrifugation, washed once with saturated barium hydroxide, then three times with distilled water, dried *in vacuo* at 60 C, and weighed. The barium soaps were then suspended in an excess of methanolic-sulfuric acid (2% acid in absolute methanol) and heated in a boiling water-bath for 30 minutes. The barium sulfate precipitate was centrifuged off, washed with boiling absolute methanol, and the washing added to the supernatant. Excess sulfuric acid was removed from the supernatant by dropwise addition of $Ba(OH)_2$, care being taken to keep on the acid side. The precipitated barium sulfate was removed by centrifugation, washed with methanol, and the washing added to the supernatant. The final supernatant was taken to dryness *in vacuo* (melting point 57 to 63 C), and the neutral equivalent determined in the

TABLE 2.—*Respective Compositions of Fractions P and S*

	Fraction P, Per Cent				Fraction S, Per Cent				
	Liver, Case 2	Liver, Case 3	Spleen, Case 1	Spleen, Case 4	Liver, Case 2	Liver, Case 3	Spleen, Case 1	Spleen, Case 4	"Bound" S Fraction
Nitrogen (micro-Kjeldahl)	1.28	1.34	1.14	1.12	3.94	4.12	4.02	3.98	4.01
Phosphorus	0.01	0.03	0.01	0.00	0.00	0.02	0.00	0.00	0.00
Reducing sugar (glucose equiv.)	32.0	36.4	31.4	33.6	23.4	22.8	24.8	24.0	23.4
Glucose/galactose ratio	3:7	3:8	3:7	3:9	2:7	2:7	2:7	2:7.5	2:7
Glucosamine	8.6	7.9	8.3	8.5	23.6	23.8	27.4	25.6	25.8
Chondrosamine	2.6	2.9	1.9	2.2	11.4	10.2	9.8	12.0	11.2
Hexuronic acids	26.8	28.2	28.4	27.9	16.4	14.8	14.2	14.6	14.2
Neuraminic acid	1.26	2.0	1.84	1.78	7.6	7.4	8.4	8.6	8.3
Fatty acids (saturated)	0.0	0.0	0.0	0.0	13.6	14.0	14.5	13.8	13.9
Sphingosine	0.00	0.00	0.00	0.00	11.6	13.4	14.2	12.8	13.5
Sulfate	6.82	7.4	3.42	5.64	1.2	0.8	....	....	0.6
Peptides (nitrogen as % total nitrogen)	4.2	....	....	....	7.8	....	11.2	....	....

usual manner. The iodine number was 42 for Fraction S (liver).

The respective compositions of Fractions P and S are presented in Table 2.

Qualitative reactions of both Fractions P and S: Ninhydrin, (weakly) positive; orcinol-sulfuric acid, deep red color; periodic acid-Schiff's reagent, strongly positive; strong iodine solution, negative.

PHYSICAL PROPERTIES

The most striking difference between Fractions P and S is the fact that, though both are soluble in water, Fraction P is insoluble in ethanol and methanol, whereas Fraction S is soluble in ethanol and aqueous methanol. This property was made use of in the separation of Fraction P from Fraction S as described in the previous sections. Both fractions were insoluble in chloroform and other nonpolar organic solvents. This property was utilized in separating lipid contaminants in the course of purification.

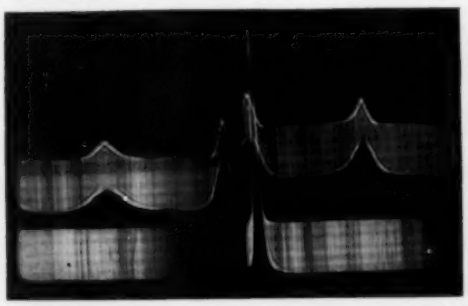
In the analytical ultracentrifuge (Spinco Model E) Fraction P sedimented very rapidly at 25,000 g, yielding a clear supernatant six minutes after attainment of full speed. Fraction S was run at 69,000 rpm for a full hour in 0.9% saline (neutralized) and proved to be inhomogeneous. The bulk of the material sedimented with S—12.6 Svedberg units, while the slowest component had S—5.2 Svedberg units.

Electrophoretic studies showed both fractions to have a net acidic charge.

Electrophoresis was performed in the Perkin-Elmer Tiselius apparatus with the use of the 2.0 ml. cell. At pH 8.6 (0.1 M diethylbarbiturate buffer) Fraction P moved completely inhomogeneously, while runs at acid pH's were impracticable because of the turbidity of the solutions. Fraction S, on the other hand, was found to migrate as a single boundary at pH 8.6 (Fig. 1), with a mobility calculated as 1.4×10^{-4} cm.^{2}/volt/sec. In phosphate buffer (0.1 M) at pH 7.0 this homogeneity was maintained, whereas in barbital (Veronal)-acetate (Michaelis buffer) buffers at 6.5 and at 5.5, it was inhomogeneous.

Osmometric measurements with a Zimm osmometer, using 2.0, 1.0, and 0.5% solutions of Fraction S, indicated a number-average molecular weight of 100,000 to

Fig. 1.—Ascending and descending electrophoretic patterns on Fraction S. Perkin-Elmer Tiselius apparatus 2.0 ml. cell. Run in 0.1 M diethylbarbiturate buffer at pH 8.68 at 110 volts, 11 ma., for 42 minutes.



120,000, on extrapolation of the $(\pi/C, C)$ curve to infinite dilution.

Because of the enormous aggregate size of Fraction P (as evident also by its sedimentation behavior), osmometric studies proved fruitless.

METACHROMASIA

The staining of material in color different from that of the dye used was called "metachromasia" by Ehrlich. This phenomenon is still poorly understood, although many of the properties that a substance must possess to produce metachromasia are now well delineated. It is now recognized that (*a*) the material has to possess a large molecular weight, or be in a state of aggregation with like molecules, so that its apparent physical properties are essentially those of a macromolecule; (*b*) it has to have a number of free acidic or basic groups which are in a charged form during the process,¶ and (*c*) the intensity of the metachromasia is in many instances directly proportional to the number of available charged groups.# The metachromasia itself, as observed histologically and proved by spectrophotometric studies of reactions in solutions, can be manifested in three ways: (1) The maximal absorption is at the same wave length as that of the dye itself, but the intensity is greatly increased; (2) the absorption maximum is still at the same wave length, but there is a secondary peak at a shorter wave length (the beta shift), or (3) the absorption maximum occurs at much shorter wave lengths than that of the dye (the gamma shift). Thus, from the histologist's point of view, either the stained material will be more intensely colored than the stained background (alpha and beta shifts), or the material will be stained in a different color from the rest of the tissue. It should be borne in mind, however, that metachromasia does not give information as to the chemical nature of the material, since such substances as nucleic acids, dextran, hyaluronic acids, and chondroitin sulfuric acid polymers all produce metachromasia with thionine, crystal violet, or toluidine blue, in spite of their widely divergent chemical constitutions. Bearing this in mind, it appeared important to establish if the metachromasia observed in the storage material in gargoylism could

¶ References 29 through 33.

References 29 and 30.

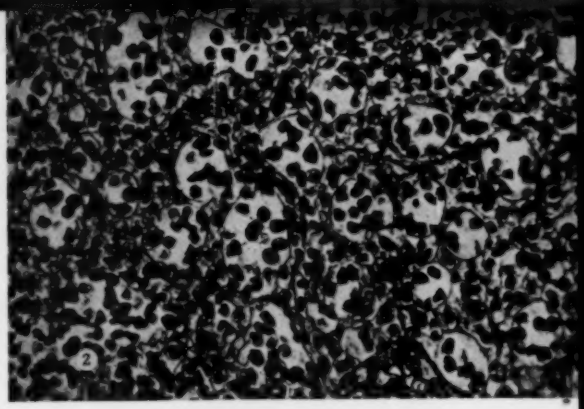


Fig. 2.—Spleen. Splenic sinusoids are dilated by the swollen endothelial lining cells, the cytoplasm of which does not take up silver. Lindsay's fixative, Wilder's stain; reduced about ½ from mag. × 600.

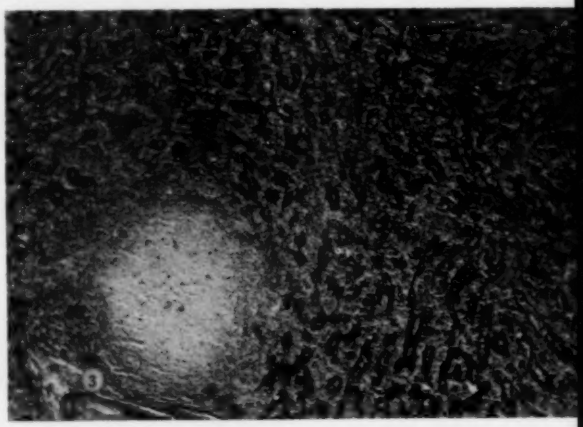
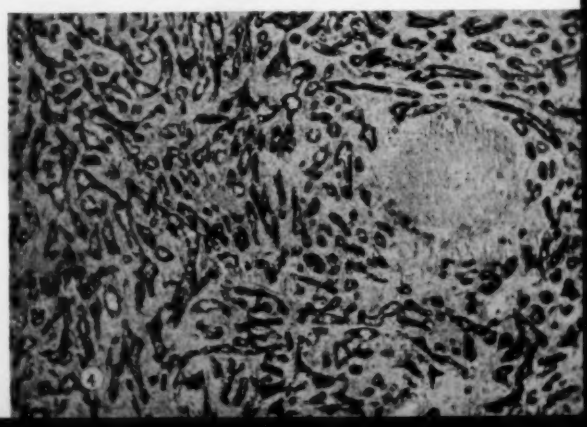


Fig. 3.—Spleen. Sinusoidal lining cells are deeply stained, while the lymphoid follicle and the rest of the pulp, which does not contain the storage substance, are not stained. Lindsay's fixative, periodic acid-Schiff stain; reduced about ½ from mag. × 120.

Fig. 4.—Spleen. Pronounced metachromatic staining of sinusoidal lining cells. Lindsay's fixative, toluidine blue O; reduced about ½ from mag. × 120.



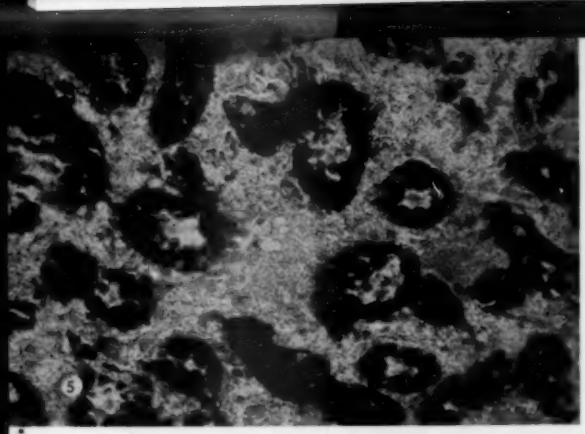


Fig. 5.—Spleen. Metachromatic staining of storage substance in sinusoidal cells. Lindsay's fixative, toluidine blue O; reduced about ½ from mag. × 600.

be traced to either or both of the isolated fractions (Figs. 2 through 5). Spectrophotometric studies were therefore made, using toluidine blue O as the dye. The choice was dictated by the fact that although this dye is far from ideal in its purity (about 70% pure), it possesses the advantages over crystal violet and thionine in that it is relatively more homogeneous than the latter two, its composition is well documented, and it does not form an insoluble precipitate on reacting with certain acidic polymers as thionine does. The results of spectrophotometric studies clearly indicated that the metachromasia observed in the storage material in tissue is also a property of the isolated material (Fig. 6). It is further evident that the spectrophotometric shifts obtained with toluidine blue are different for Fractions P and S. Fraction S produces an increase in the absorption at the same wave length as the absorption maximum of toluidine blue, with an enhancement of the absorption of the secondary peak at 600 mμ. On the other hand, Fraction P produces a marked metachromatic shift to a shorter wave length (gamma shift), so that the absorption maximum is now at 545 mμ and similar to the metachromasia produced by heparin.

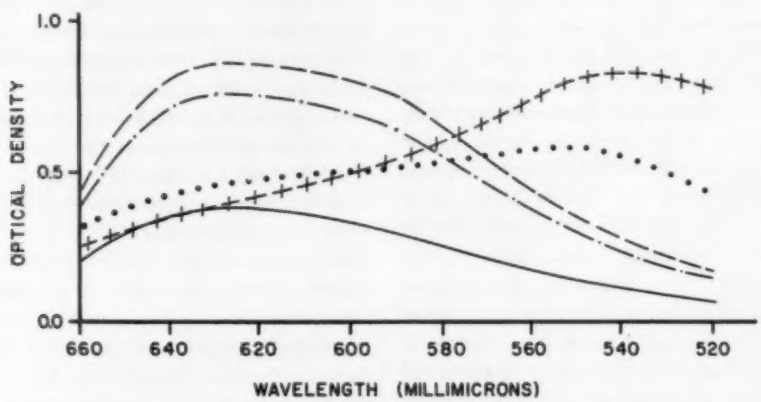


Fig. 6.—Spectrophotometric evidence of metachromasia of isolated storage substance. ———— indicates 0.00164% toluidine blue O (69% dye); ——·——·——, 0.0328% Fraction S in 0.00164% toluidine blue O; — — — —, 0.0984% Fraction S in 0.00164% toluidine blue O; ·····, 0.0984% Fraction P in 0.00164% toluidine blue O; ++++++++, 0.075% heparin in 0.00164% toluidine blue O. Solvent, 0.05 M phosphate buffer pH 6.82.

COMMENT

A close parallelism between the histopathological characteristics of the "storage substance" in storage diseases in general and the properties of the isolated storage material is usually not expected. The reason for this has been ascribed [35] to the complex organization that the stored material usually undergoes within the cell in terms of "bonds" of varying stability formed between the storage substance and cytoplasmic proteins and other macromolecular constituents (lipids, fibrillar proteins, etc). It is therefore gratifying to find that the properties of Fractions P and S, as here described, fully correspond to the solubility characteristics of the storage

substance in gargoylism, i. e., solubility in formaldehyde, aqueous ethanol, and water. The staining characteristics (Figs. 3, 4, and 5) of the material (PAS, toluidine blue) is satisfactorily explained by the polysaccharide constituents of both Fractions P and S (Table 2), while the metachromasia observed histologically is duplicated in vitro by Fraction P (Fig. 6), presumably by virtue of its sulfonated polysaccharide character. It seems therefore reasonable to assume that the storage material in gargoylism represents the accumulation of two distinct substances, of which one can be regarded as belonging to the class of water-soluble glycolipids (Fraction S) and the other as a sulfonated polysaccharide or mucopolysaccharide (Fraction P). The proportion in which these two substances occur may, however, vary in different organs and in different cases, as judged from the differences in yields (Table 1). The variation observed in the cases recorded in this study is perhaps responsible for the duality ascribed to the storage material by some investigators. Dawson [14] pointed out that the solubility characteristics of the material found in the nervous system differed from that observed in visceral organs, the former being more germane to the histochemical behavior of cerebrosides. This observation coincides with our own experience. Similarly, the morphological similarity between the involved nerve cells in Tay-Sachs' disease and those in gargoylism has been repeatedly commented upon.* The dual nature of the storage material may also serve to explain the conflicting reports of Brante.† Thus, his histochemical studies led him to believe that gangliosides were responsible (hence, a glycolipid) for the character of the storage material, while his isolation procedure on the liver yielded a mucopolysaccharide. Though the sulfate content of his mucopolysaccharide is much higher than that of any of our preparations, the variation of sulfate in our different sulfonated polysaccharide preparations (Fraction P, Table 2) indicates that this may well be a characteristic of this fraction, and that variations in the degree of sulfonation in different cases should be expected. In view of the duality of the chemical nature of the storage substance, one is inclined to regard the basic metabolic genetic defect operative in gargoylism as being directly responsible for the accumulation of one of these substances, while the second is present as a consequence of the interference of normal function in involved cells as a result of the deposition of the first. An analogy to this view is found in the increment in all lipid fractions in lipid-storage diseases of the Gaucher and Niemann-Pick types, although a specific increase of cerebrosides and sphingomyelins, respectively, characterizes these conditions.

If a similar scheme of events is operative in gargoylism, the generalized involvement of all tissues directly derived from primordial mesenchyme (connective tissue, cartilage, bone, leucocytes, tunica propria of the cornea, sinusoidal cells of lung, liver, and spleen) points to an abnormality of structural polysaccharide metabolism as the primary defect. It could thus be visualized that the inability of mesenchyme-derived tissue to metabolize structural polysaccharides in proportion to protein growth and differentiation leads to morphological anomalies in structural systems normally rich in polysaccharides, resulting in the formation of abnormal connective tissue, reticulum tissue,[24] cartilage, valvular tissue of the heart, and Bowman's layer of the cornea. The clinical appearance of "dysostosis multiplex" or "lipochondrodystrophy" would thus be the expression of the abnormalities of development of cartilage and failure of its transition to normal bone. The reason for the inguinal and abdominal hernias, cloudiness of the corneas, cardiac dilatation with subsequent failure, and hepatosplenomegaly would similarly find explanation in spite of the seeming lack of relation of these systems to the skeletal deformities. It is noteworthy that the hepatosplenomegaly develops as a result of the deposition of the storage material pri-

* References 4 and 14 through 16.

† References 19 and 20.

marily in lining cells of sinusoids in both organs, further emphasizing the seat of the metabolic defect as being in mesenchyme-derived tissue. Muscles are generally spared. In view of the histochemical studies of Lindsay and his associates,[1] showing highly metachromatic granules in the cornea, one can assume that corneal cloudiness in this disease is the direct outcome of the accumulation of large amounts of Fraction P in the tunica propria. Since, however, corneal opacities are a late manifestation of the disease, one cannot assume from this alone that disturbance in polysaccharide metabolism is the primary defect. Furthermore, the question of whether Fractions P and S represent products of abnormal metabolism, or whether they represent normal tissue constituents, commonly present in all tissues in small amounts but which accumulate in gargoylism because of a genetically determined deficiency of enzymes, cannot be answered. An answer to this question might be sought, however, in the high proportion of patients with this disease also having Alder bodies in their leucocytes. Since metachromatic stippling of leucocytes is known to be a genetically transmitted stigma in otherwise healthy persons,‡ this instance may represent the accumulation of Fraction P in a very restricted element of mesenchyme-derived tissue, i. e., leucocytes. One would thus be inclined to regard the accumulation of Fraction P as the primary manifestation of the metabolic anomaly in gargoylism and to define the disease as a genetically induced, generalized abnormality in the metabolism of structural polysaccharides.

SUMMARY AND CONCLUSIONS

The "storage substance" was isolated from the surgically removed spleens of two patients with gargoylism and from the spleens and livers obtained at autopsy from two other patients. The material was shown to consist of a mixture of two distinct chemical entities. One of these is a complex polysaccharide having glucose, galactose, hexosamines, and sulfate as its constituents. It was found to be soluble in water and formaldehyde, insoluble in ethanol, methanol, and other organic solvents. It also showed the metachromasia with toluidine blue which is characteristic of the storage substance in involved tissues of patients with this disease. The second component of the storage substance was shown to be a water-soluble glycolipid, soluble in ethanol but insoluble in other organic solvents. It was electrophoretically homogeneous at pH 8.6 and 7.0, but inhomogeneous at lower levels of pH. Fatty acids, sphingosine, neuraminic acid, hexuronic acids, hexosamines, glucose, and galactose were shown to be its chief constituents.

The chemical pathology of gargoylism in the light of these findings is discussed. It is concluded that gargoylism represents a genetically induced defect in the metabolism of structural polysaccharides.

Dr. Janine Kint, Miss Martha Quinn, and Miss Marilyn Rumley gave valuable assistance in later phases of this work. Dr. Benjamin H. Landing made the histological sections.

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Thrombosis of Hepatic Veins

The Budd-Chiari Syndrome

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Thrombosis of hepatic veins, more commonly called Budd-Chiari syndrome, was first described by Budd * in his book "Disease of the Liver," published in 1846. Detailed studies were not made until half a century later, when Chiari [2] reported three autopsy cases; he believed that the thrombosis was due to thrombophlebitis of the hepatic veins.

Thompson,[3] reviewing this subject in 1947, mentioned that there were only 95 cases reported up to that time. Of all the cases reviewed, the youngest patient was 17 months and the oldest was 61 years, but the majority of cases occurred between 20 and 30 years of age. Among the etiologic factors, hepatic suppuration, tumor metastasis, phlebitis of hepatic veins, and peritonitis have been mentioned. Other contributory factors were polycythemia vera, pregnancy, and leukemia. In recent years several etiologic factors have been recognized. Selzer and co-workers [4] had reported the association of a species of toxic plant, Senecio, with thrombosis of hepatic veins in 12 cases among three families. Dodd, Johansmann, and Rapoport [5] pointed out the possible relationship between Chiari's syndrome and nephrosis in a child. They postulated that an increase in the globulin brings about a greatly increased serum accelerator factor, with possible increase of fibrinogen.

Among other possible causes the following theories can be mentioned: (1) extending obliterative process from the ductus venosus to the hepatic vein, as proposed by Rolleston and McNee,[6] but this can only explain the syndrome when it occurs in infancy; (2) the theory of congenital venous abnormality; (3) fibrous bands occluding the ostium of main hepatic veins, as suggested by Beattie and Hildebrand [7]; (4) recurrent idiopathic thrombophlebitis [8]; (5) trauma to the hepatic vein, as in whooping cough; (6) thrombosis of the hepatic veins associated with generalized vascular disease.[9] The last condition is rare. Because of the divergence of etiologic factors in the Budd-Chiari syndrome, we report a case in which the thrombosis of hepatic veins and other vessels is primarily due to vascular changes.

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* Cited by Hoover.[1]

REPORT OF A CASE

History.—A 29-year-old unmarried woman, of Greek ancestry, was admitted to the Illinois Masonic Hospital on Oct. 5, 1953, with anorexia and fever present 10 days before admission. There was pain in the right shoulder, epigastrium, and right lower quadrant, but the nature of it was not described. There was no nausea, vomiting, diarrhea, or constipation. She had no known exposure to chemicals and did not receive any transfusion or vaccination. The family history was irrelevant. Positive findings on admission included elevated temperature, 101.4 F; slightly hypertrophied tonsils with prominent scars; soft systolic murmur at both mitral and aortic areas; a blood pressure of 150/90 mm. Hg, and tachycardia (heart rate of 100 per minute) with normal sinus rhythm; questionable palpable spleen but markedly enlarged liver, 4 fingerbreadths below the right costal margin, with a smooth and soft edge. There was no jaundice of the skin or sclerae. Laboratory findings showed RBC 4,640,000 per cubic millimeter; Hgb. 13.4 gm. per 100 ml., and WBC 18,000 per cubic millimeter with 85% segmented forms. The platelets were 300,000 per cubic millimeter. Urine had a specific gravity of 1.010 and contained 5 mg. per 100 cc. protein, but no sugar. The urobilinogen was 0.35 E. U. Blood Kahn test was negative. X-ray studies revealed suggestive left ventricular enlargement and suspected areas of inflammatory infiltration

and associated pleuritis of right lung, but the latter finding was not conclusive. Electrocardiogram was interpreted as demonstrating left axis deviation, sinus tachycardia, and suggestive left heart strain with slight counterclockwise rotation but no evidence of recent or old infarct.

Hospital Course.—The patient had intermittent fever (temperature ranging from 99.2 to 102.8 F), and the pulse rate remained around 120 per minute. The blood pressure varied from 140 to 160 mm. Hg systolic and 90 to 108 diastolic. She was treated with multiple vitamins, parenteral fluid, and oxytetracycline (Terramycin) 350 mg. t. i. d., and later was digitalized. Dioxyline phosphate (Paveril) and mersalyl and theophylline (Salyrgan) were also given, with no apparent improvement. Four days later, scleral jaundice was noticed and diarrhea developed, probably due to oxytetracycline. Blood chemistry studies done at this time revealed direct and indirect bilirubin of 1.5 and 3.7 mg. per 100 cc., respectively, thymol turbidity 7.5 units, and cephalin-cholesterol flocculation 3+ in 24 hours. The prothrombin time was 20 seconds (control, 13 seconds); the albumin-globulin ratio, 3.19: 3.5 gm. per 100 cc. Three days latter, possible fluid accumulation in the abdominal and chest cavities was noted, but the liver was described as decreasing in size. Repeated hemogram showed RBC 4,310,000, Hgb. 13 gm. per 100 ml., and WBC 12,850 with 88% segmented forms. The repeated blood chemistry studies revealed the total bilirubin dropped to 2.6 mg. per 100 cc., with direct and indirect 1.3 mg. per 100 cc. each, whereas the thymol turbidity and cephalin-cholesterol flocculation remained the same. On the 11th hospital day about 4 liters of straw-colored and slightly cloudy fluid was removed by paracentesis. It had a specific gravity of 1.014 and was negative for tumor cells, acid-fast bacilli, and other bacteria. Four days later, deep-seated hemorrhages were found in the feet and the hypothenar eminence of both hands. The value for alkaline phosphatase done that day was 6.71 Bodansky units. Later, crepitant rales developed at the left base, and the condition of the patient deteriorated. She died 18 days after admission in a deep comatose condition, about four weeks after the onset of the first symptoms. An autopsy was performed two hours after death. Only the significant findings are given.

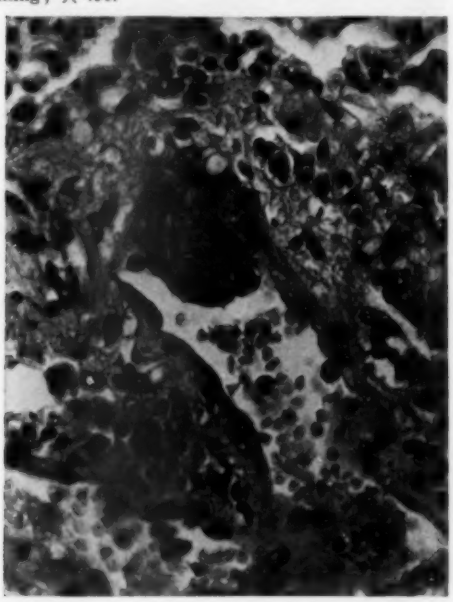
The body was that of a well-developed and well-nourished white woman. The skin and sclerae had slight jaundice. In the adipose tissue at the lower end of the sternum there were recent hemorrhages, the largest 3 cm. in diameter. The abdominal cavity contained 2000 cc. of slightly turbid yellowish fluid with a specific gravity of 1.014, and the right chest cavity contained 50 ml. of clear yellowish fluid. The heart weighed 210 gm. and showed a large, well-healed grayish-white scar, measuring 5 cm. in diameter, in the posterior wall of the left ventricle and posterior half of the septum. The endocardium of the posterior wall of the left ventricle had slight fibrosis and scattered fresh hemorrhagic areas. There was only moderate narrowing of the left anterior descending and the right coronary arteries, but in the left circumflex artery the lumen was almost completely obliterated. The valves were not remarkable. The lungs showed marginal atelectasis over the right lower lobe and several small subpleural hemorrhages, 1 to 2 cm. in diameter. The spleen weighed 260 gm. and was slightly firmer in consistency, with definite increase of trabeculation. The Malpighian corpuscles were inconspicuous. No thrombosis of splenic artery or vein was present. The liver weighed 2750 gm. and had a dark purplish-red surface with irregular light-brownish mottling. The sectioned surface was in general extremely congested, oozing a moderate amount of purplish-red blood with irregular small to large dark-reddish areas surrounded by tan to light-yellowish zones. However, the consistency was uniform and soft. In the right lobe the large hepatic veins were occluded by freshly formed thrombi. These, for the most part, could be easily detached from the intimal surface, but some were slightly adherent. The thrombi had propagated into the lumen of the inferior vena cava to a point just below the right atrium. The wall of the gall bladder measured 6 to 7 mm. in thickness and appeared fibrotic. There was a solitary mulberry-type stone, which had completely occupied the cavity. However, the remaining biliary passage was not remarkable. The right adrenal was enlarged to 10×6×5 cm. and contained a large amount of dark-reddish clotted blood; on section a very small amount of normal adrenal tissue was recognizable. The left adrenal was not remarkable. Each kidney weighed 150 gm., and the surfaces presented numerous minute fleabitten type petechial hemorrhages. Permission was not granted to examine the cranial contents.

Microscopic Findings.—The heart showed extravasation of blood in the pericardial adipose tissue. The posterior wall of the left ventricle had a healed infarct and intimal fibrous thickening of the small arteries. There was fibrous thickening of the endocardium but no hypertrophy of the myocardial fibers. The wall of the left circumflex artery displayed marked intimal fibrous thickening which was not uniform throughout the whole circumference (Fig. 1), causing only partial occlusion. In the deeper intima and media there were vascularation and slight cellular infiltration with lymphocytes, histiocytes, and

Fig. 1.—Left circumflex artery, low magnification, showing marked intimal thickening with partial occlusion of lumen; × 35.

a few polymorphonuclear leucocytes. At one point in the media the continuity of smooth muscle fibers was interrupted by degenera-

Fig. 2.—Small pulmonary vessel, high magnification, showing focal fibrinoid subendothelial thickening; × 450.



tive changes with basophilic staining. The lungs revealed recent embolus with no organization in a large branch of the pulmonary artery. The pulmonary arterioles showed focal fibrinoid masses protruding into the lumen underneath the intact endothelial lining (Fig. 2). Fresh hemorrhage was present in the adjacent alveoli. The liver presented a variety of findings. In some areas there was marked congestion of the central veins and sinusoids with destruction of the liver cells

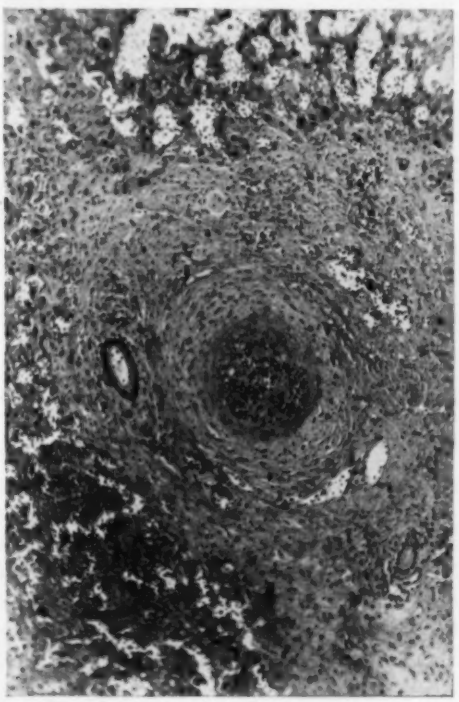


Fig. 3.—Medium-sized hepatic vein, low magnification, showing intimal proliferation and organized thrombus with recanalization. Note the marked dilatation and congestion of sinusoids; × 100.

resulting in narrowing and irregularity of liver cords. In other areas, there was complete disappearance of liver cells, which were replaced by early fibrosis with young fibroblasts, rich vascularity, a few hemosiderin-containing phagocytes, and a few remaining bile ducts, but no cellular infiltration. In other areas, there was complete coagulation necrosis with acute inflammatory reaction. The small- and medium-sized hepatic veins

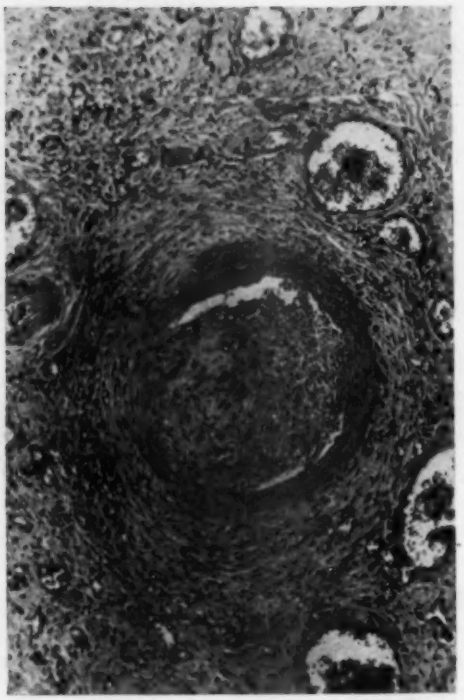
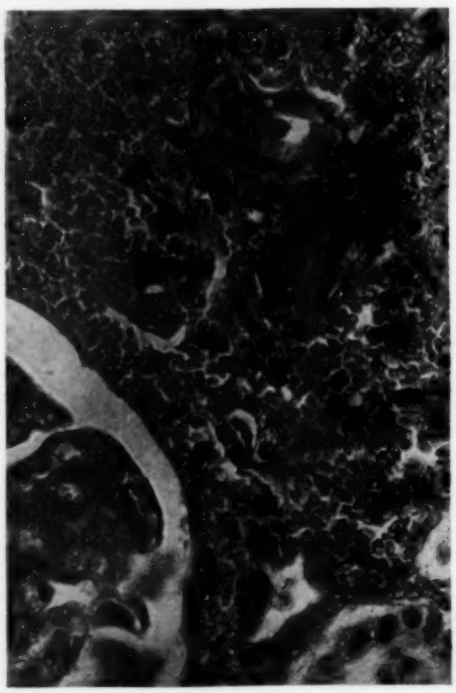


Fig. 4.—Serosa of gall bladder, low magnification, showing occlusion of small artery by endothelial proliferation. Note also the marked congestion of small veins. × 100.

showed considerable intimal thickening and proliferation of endothelial cells which anastomosed freely in the lumen, forming false "compartments." The lumina of many medium-sized hepatic veins were occluded either by marked endothelial proliferation or by actual thrombus of varying ages (Fig. 3). The main hepatic veins were completely occluded by fresh thrombus, in which slight early organization was already present. The wall of the main hepatic veins was infiltrated with mononuclear cells and polymorphonuclear leucocytes. The gall bladder revealed marked chronic inflammatory reaction with lymphocytic infiltration and fibrous thickening of the submucosa and muscularis. The mucosal epithelium was intact. The small arteries in the serosal fibroadipose tissue showed fibrosis and small round-cell infiltration of the adventitia, interruption of the continuity of the media, intimal fibrous thickening, and endothelial proliferation. Some of the lumina were occluded by either fresh or old organized thrombus, but, in a few others, the occlusion was due to proliferation of endothelial cells only (Fig. 4). There was also congestion of the capillaries and small veins adjacent to the thrombosed vessels. The small intestine showed chronic inflammatory reaction of the adventitia where the small arteries displayed marked thickening of the wall with hypertrophy of the media and intimal proliferation. The right adrenal showed hemorrhagic infarct in addition to fibrosis and chronic inflammatory reaction of the cortex as well as the medulla. In the periadrenal fibroadipose tissue the small arteries displayed similar changes, i. e., intimal fibrous thickening and endothelial proliferation. A few showed occlusion of the lumen with partially organized thrombus and chronic inflammatory reaction of the wall. The kidney revealed marked fibrinoid change of many arterioles whose lumina were completely oc-

Fig. 5.—Kidney, high magnification, showing the occlusion of the arteriole by fibrinoid material. Note also the fresh hemorrhage in the tubules; × 450.



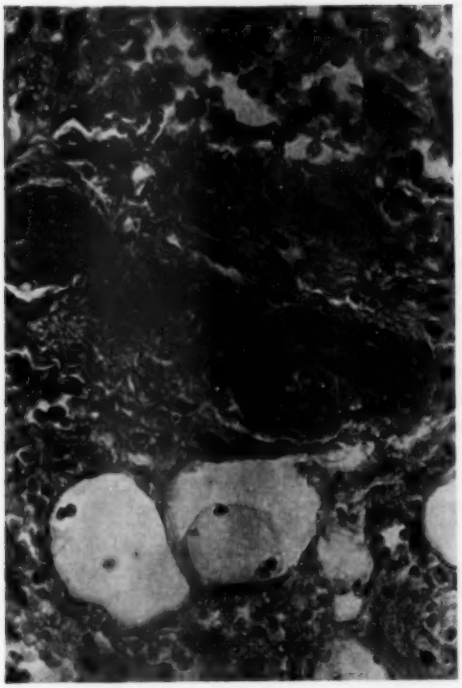


Fig. 6.—Peristernal fibroadipose tissue, high magnification, showing the occlusion of arteriole by fibrinoid substance; × 450.

cluded by acidophilic-staining fibrinoid substance (Fig. 5). The adjacent glomeruli had congested capillary tufts, and the surrounding tubules were dilated and contained fresh blood. The bone marrow had the usual cellularity, with an increase of megakaryocytes. In several areas the cells were poorly stained, suggestive of karyorrhexis. In the peristernal fibroadipose tissue there was fibrosis, chronic inflammatory reaction, and thrombosis of small vessels, both arteries and veins. The small arteries had intimal thickening and marked endothelial proliferation which nearly filled up the whole lumen. In a few arterioles there was, in addition, fibrinoid change with complete occlusion of the lumen (Fig. 6).

COMMENT

The Budd-Chiari syndrome is now considered to be due to a variety of causes with only one thing in common, i. e., the thrombosis of hepatic veins. The patient reported here presents three definite features. First, the immediate cause of death was rapid hepatic failure from the thrombosis of hepatic veins producing recent and fresh destruction of liver cells. Second, the thrombosis involved both arteries and veins in subcutaneous tissue as well as internal organs beside the liver. Third, there was both old and recent vascular damage, with destruction of walls like that seen in periarteritis nodosa.

Widespread vascular disorder associated with Budd-Chiari syndrome has been reported by Hirsh and Manchester [10] and, more recently, by Gerber and Mendlowitz.[11] The last authors noted that three of the five reported cases had thrombocytopenia. Thrombocytopenia is also known to be associated with another form of vascular thrombosis, the thrombotic thrombocytopenic purpura, described first by Moschcowitz [12] and later by Baehr and co-workers.[13] In the present case, no thrombocytopenia was observed in one determination.

Histologically, this case presents very interesting and significant findings. There were changes involving vessels of varying sizes in different organs. Damage to the large artery is exemplified by the left circumflex coronary artery, which had marked, but not uniform, subintimal fibrous thickening causing partial occlusion of the lumen. The media at one point showed interruption of continuity by degenerative change with basophilic staining. The medium- and small-sized arteries, as seen in the fibroadipose tissue of the serosa of gall bladder and small intestine and right adrenal, had either intimal thickening or proliferation of endothelial cells, or both. Many of them showed occlusion of the lumen by either the proliferative endothelial cells or thrombus. A few of the medium-sized arteries, in addition, displayed interruption of the continuity of media and fibrous thickening of the adventitia, which was infiltrated with a few lymphocytes. The arterioles, seen mainly in the kidneys, lungs, and subcutaneous tissue, had diffuse or focal fibrinoid swelling completely or partially occluding the lumen. This occlusion of lumen of arterioles by fibrinoid swelling, together with the hemorrhage into the lumen of the tubules and

congestion of the glomerular tufts, undoubtedly was the cause of the flea-bitten appearance of the kidneys.

The small- and medium-sized hepatic veins had the similar changes of endothelial proliferation and fibrous intimal thickening, which undoubtedly lead to thrombosis. The thrombus later propagated to the larger and, finally, the main hepatic veins. The pathological findings of the liver can be well correlated with the vascular changes. The complete disappearance of hepatic cells replaced by fibrous connective tissue was certainly due to thrombosis by old organized thrombus of the small- and medium-sized hepatic veins, whereas the marked acute congestion and coagulation necrosis were the result of propagation of the thrombus into the main hepatic vein, causing complete occlusion of the latter, with final hepatic failure and death of the patient.

CONCLUSION AND SUMMARY

A case of Budd-Chiari syndrome with thrombosis of hepatic veins is reported. The patient died in rapid hepatic failure.

The vascular changes of the liver were only a part of the generalized vascular pathologic change.

The thrombosis of the vessels involved both arteries and veins of various sizes in subcutaneous tissue as well as in other internal organs beside the liver.

The vascular changes were of varying ages, and the etiology of these changes is undetermined.

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Estimation of Survival Time Following Injury

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and
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In military medicine, as well as in civilian forensic medicine, it is frequently of paramount importance to determine how long the patient lived after sustaining a fatal injury. Pathologists have been able to calculate the survival time with some degree of accuracy by gross and microscopic examination of the injured tissues. In many cases the mere dimensions of the wound or wounds and the organs involved reveal that death was instantaneous. Decapitation, large defects in the heart and great vessels, and massive mutilations are incompatible with life. On the other hand, even in the presence of severe trauma to the brain or injuries involving the heart and other vital organs, survival times of hours or days may be reported.

Evacuation of the wounded from the battlefield reached a high degree of efficiency during the Korean conflict. Yet, in this survey there were many casualties classed as killed in action (KIA) in which the agonal period unquestionably had been of considerable duration. In an attempt to establish the survival time following injury, the authors collected information to fill in the following outline:

(*a*) Type of wound and organ or organs involved
(*b*) Estimation of amount of hemorrhage
(*c*) Reaction of the tissue at the site of injury
(*d*) Secondary complications following injury, such as development of pneumonia or lower nephron nephrosis
(*e*) Histologic appearance of the spleen, liver, and adrenal glands

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Arlington Hospital (Dr. Enos).

Fifty-one cases with known survival times were acceptable. This series consisted of 16 suicides, 1 homicide, 18 accidental deaths, and 16 Korean combat casualties. The selection was restricted to those cases in which autopsy protocols furnished a definite survival time and tissues for microscopic examination were available.

It is well known that unless death is almost instantaneous a series of reactions occur at the site of injury which are called by the collective term "aseptic inflammation." [1] These reactions are first manifested by capillary dilatation, with margination and emigration of leucocytes occurring within a few minutes of the trauma. It takes approximately one hour for any appreciable number of leucocytes to migrate. Cellular infiltrate is well marked by 13 to 18 hours after injury. Diffuse extravasation of blood into the tissue interspaces also suggests life after injury.

These local reactions may be modified in war wounds because of the extent of tissue damage. Disruption of the local circulation, vascular spasm, and shock all interfere with the phenomenon of aseptic inflammation and the extravasation of blood into the tissue spaces.

In war wounds it is often impossible to determine the amount of hemorrhage which has occurred, for the examining medical officer does not see the casualty at the scene of injury. Furthermore, a postmortem flow of blood frequently occurs at the wound site while the body is being moved during the first few hours after death.

The reaction of tissue not directly injured is also helpful in determining the possibility of life after injury. The development of early bronchopneumonia suggests an agonal period of several hours' duration. A reaction of this type, however, is influenced by many variables such as age, environment, and the general health of the victim.

Of particular significance are the morphologic changes found in the spleen, liver, and adrenal glands.

THE SPLEEN

Klemperer [2] directed attention to the fact that in a majority of instances the normal spleen contains a large number of eosinophiles. Allen [3] reemphasized this finding by studying the spleens in 200 cases, both those of sudden death and those in which there was a known survival time. He noted a conspicuously large number of eosinophilic leucocytes in the spleen of 94% of patients dying immediately after coronary occlusion before infarcts had developed in the heart and in 81% of spleens from patients whose death was sudden and violent. Eosinophiles were observed in the splenic parenchyma in only 9% of routine unselected cases. He further demonstrated that the eosinophilic leucocytes tended to disappear from the spleen as the period of survival following the accident was prolonged.

The incidence of eosinophilia of the spleen in cases of accidental death in our series approached 80%. However, when death was preceded by a stress situation such as combat the incidence fell to 30%. The incidence of eosinophilia in suicides was not so high as in accidental deaths. It approached 50%.

In cases of sudden accidental death the parenchyma of the spleen contained 10 to 20 eosinophiles per high-power field. If the agonal period after injury was more than six hours, no eosinophiles were found in the splenic parenchyma. The observation of one or two eosinophiles per high-power field in a congested spleen would suggest the possibility of an agonal period of 30 minutes to 5 hours.

THE LIVER

The presence of large amounts of glycogen in the liver cell cytoplasm causes it to appear pale and fluffy (Fig. 1*A*). Cells of this type were seen in 57% of all cases of sudden death, including battle injuries, suicides, homicides, and accidental deaths. If the agonal period lasted more than 24 hours, all the hepatic elements lost their fluffiness and appeared as opaque, darkly acidophilic cells (Fig. 1*B*). There were no distinct stages in the change from the fluffy cell to the opaque cell, although cells of both types were encountered in many cases with agonal periods lasting from 5 to 12 hours. The transformation from the fluffy cell to the opaque cell is thought to be due to loss of glycogen during the agonal period as well as to the postmortem loss of liver glycogen resulting from continuing glycolysis in the liver after death.

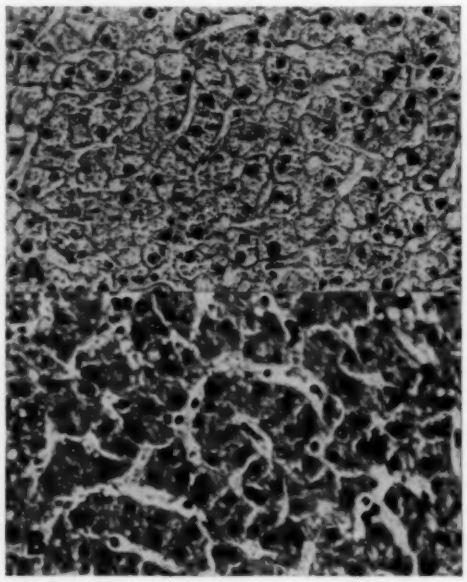

Fig. 1.—*A*, liver from sudden combat death with zero survival time; liver cells filled with glycogen. *B*, liver from case with a survival time in excess of 48 hours, no intravenous therapy; liver cells darkly acidophilic. Reduced about ⅙ from mag. × 275.

Popper [4] has made the observation that spaces between the liver cell cords and the sinusoids are, as a rule, completely obliterated in sudden accidental death but are usually wide open after an agonal period of 10 minutes. In our series there was general agreement with this observation, but the possibility that this feature may also be influenced by postmortem changes in the liver was given consideration.

ADRENALS

The cells of the zona glomerulosa and the zona fasciculata in a healthy young person exhibit a "watery vacuolated cytoplasm" owing to the presence of lipids (Fig. 2*A*). The cells of the zona reticularis are usually more homogeneous than those of the other zones and frequently contain pigment. If the agonal period following severe trauma lasts from two and one-half to seven days, the cells of the three zones frequently lose their vacuolated appearance and display a condensed homogeneous acidophilic cytoplasm (Fig. 2*B*). This picture is interpreted as lipid depletion. The adrenal medulla of low activity is composed of small dark-staining cells, whereas the normal adrenal medulla is composed of larger cells with lighter-staining nuclei and displays acidophilic globules in the secretory canals.[5] In several cases the cells of the medulla presented evidence of exhaustion by the end of six to seven days.

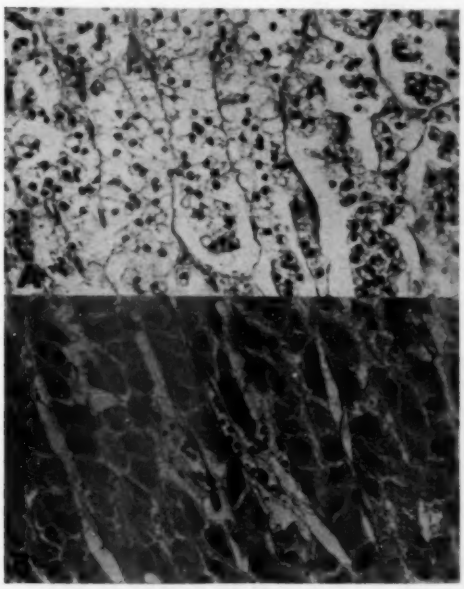

Fig. 2.—*A*, adrenal cortex from case of sudden death with zero survival time; cortical cells exhibit watery, vacuolated cytoplasm. *B*, adrenal cortex from case with survival time in excess of two and one-half days; lipid depletion of the cytoplasm. Reduced about ⅕ from mag. × 275.

COMMENT

The histologic changes found in the spleen, liver, and adrenal glands, which vary according to the length of survival time, are presumably a morphologic manifestation of the general adaptation syndrome as described by Selye.[6] Both the adrenal corticoids and epinephrine are activated during the stress, and these stubstances cause physiologic changes in the spleen, liver, and adrenals which are manifested morphologically.

Disappearance from the spleen of eosinophiles is probably related to the hypothalamus pituitary-adrenal chain of epinephrine eosinopenia. Epinephrine as well as the corticoids affect glycogen metabolism. Following the administration of epinephrine hydrochloride intramuscularly the blood sugar concentration normally increases 35 to 45 mg. per 100 cc. in 45 minutes to 1 hour,[7] and this sugar is supplied by the liver. Selye[6] has shown experimentally that overdosage of cortisone in the rat causes morphologic changes in the liver. He further points out that in the phase of countershock hyperglycemia is one of the defenses against shock. This is brought about by the discharge of corticoids into the blood. All these physiologic changes are reflected histologically by glycogen depletion of the liver. The cellular changes in the adrenals are thought to be an indication of exhaustion.

CONCLUSION

The morphologic appearance of the spleen, liver, and adrenal glands or any combination of these organs is helpful in many instances in determining survival time. With a thorough gross and microscopic study of the injured tissues as well as a microscopic study of the spleen, liver, and adrenal glands, a pathologist can estimate survival time with a fair degree of accuracy in most cases.

SUMMARY

The length of life following trauma can be determined by investigation at the scene of

accident and by gross and microscopic studies of the body tissues.

Injured tissues undergo gross and microscopic changes which indicate length of survival after trauma.

Microscopic changes in the liver, spleen, and adrenal glands vary according to the length of survival time and suggest the duration of the agonal period.

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News and Comment

PERSONAL

Lederle Medical Faculty Award to Dr. Albert Hand.—Dr. Albert Hand, of the Department of Pathology, University of Tennessee, is one of the recipients of the 1955 Lederle Medical Faculty Awards.

Appointment of Capt. William M. Silliphant.—Capt. William M. Silliphant (MC), U. S. N., has been appointed The Director, Armed Forces Institute of Pathology, beginning Aug. 1, 1955. Captain Silliphant succeeds Brig. Gen. Elbert DeCoursey, who will become Commandant of the Medical Field Service School, at Brooke Army Medical Center, San Antonio, Texas.

Captain Silliphant has been Deputy Director of the AFIP since February, 1952, He received his M.D. degree from Stanford University School of Medicine, in 1931, and has been in the U. S. Navy since that time, having served at various naval hospitals, both in the United States and overseas. In 1953 Captain Silliphant served as Pathologist on a Wound Ballistics Research Team, and he has been particularly interested in the study of the influence of body armor on war wounds.

He is a Specialist certified by the American Board of Pathology and a Fellow of the American Medical Association, the American College of Physicians, the American Association of Pathologists and Bacteriologists, and the American Society of Clinical Pathologists. He was awarded the Navy Letter of Commendation with Commendation Ribbon by the Secretary of the Navy for distinguished service while a prisoner of war. He was also awarded the Army Distinguished Unit Badge, Philippine Defense 1941-1942, and, by the Philippine Government, the ribbons of Philippine Defense, Philippine Liberation, and Philippine Independence.

The assignment as Director of AFIP is normally for four years and rotates in order among the Army, Navy, and Air Force.

Mixed Mesenchymal Sarcoma of the Corpus Uteri

LT. ROBERT L. ALZNAUER (MC), U.S.N.R.

It is the purpose of this paper to present an autopsied case of mixed mesenchymal sarcoma of the corpus uteri and to review and analyze all similar autopsied cases reported in the literature. A concept of histogenesis based on such information and data as are now available in the literature will be presented, and the relationship of the mixed mesenchymal sarcoma and the "heterologous" carcinosarcoma[1] (i. e., "combined mesenchymal sarcoma and carcinoma"[2]) of the corpus uteri will be discussed.

The clinical picture in these cases has been comprehensively covered by Liebow and Tennant,[3] Hartfall,[4] and Lebowich and Ehrlich.[5] It is not within the scope of this paper to review all of the other recorded cases of mixed mesenchymal sarcoma of the corpus uteri in which an autopsy was not performed. Furthermore, the review and analysis to be presented will not include the autopsied cases of either the "heterologous" carcinosarcoma of the corpus uteri or the mixed mesenchymal sarcoma of the cervix uteri and vagina (the so-called "sarcoma botryoides").

Grossly, the mixed mesenchymal sarcoma, or "heterologous" endometrial sarcoma,[6] is a polypoid tumor which projects from the endometrial lining into the uterine cavity. In addition to cellular sarcoma of anaplastic type, its histologic components include differentiated mesenchymal tissues which are heterotopic to the uterus. These most commonly consist of rhabdomyoblasts and cartilage. The rhabdomyoblasts which are well differentiated contain cross striations. In the more primitive ones, however, cross striations may not be demonstrable. The islands of cartilage invariably show the histologic characteristics of chondrosarcoma. In addition, osteoid, bone, and fat have been found in a few neoplasms. The mixed mesenchymal sarcoma, however, does not contain an intrinsic adenocarcinomatous component.

Although the sites of dissemination are quite variable, they can be divided into the following three groups: (1) In some cases there is only a recurrent pelvic tumor without distant metastases; (2) in other cases there are only distant metastases without a local pelvic recurrence, and (3) in still other cases there are both distant metastases and a recurrent pelvic tumor (Table). In some cases there are also scattered peritoneal implants in addition to the recurrent pelvic mass. As might be expected, the distant metastases most frequently involve the lungs. To a less extent they also involve the liver, lymph nodes, and bone. As will be shown, there may be considerable variation between the histologic composition of the metastases and that of the primary neoplasm in some of the cases.

In its biologic behavior the mixed mesenchymal sarcoma is a highly malignant tumor, even though in many cases the neoplasm

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Lt. Alznauer has returned to civilian practice and is at present Assistant Pathologist, Mount Zion Hospital, San Francisco.

The opinions or assertions contained herein are the private ones of the writer, and are not to be construed as official or reflecting the view of the Navy Department or the Naval Service at large.

Autopsied Cases of Mixed Mesenchymal Sarcoma of the Corpus Uteri

Case Source *	Age	Race	Pertinent Clinical Information	Death	Primary Neoplasm †	Pelvic Recurrence †	Peritoneal Implants †	Distant Metastases †			
								Lungs & Pleura	Liver	Lymph Nodes	Miscellaneous
1. Wagner, 1854	55	W	None available	Death probably due to peritonitis	C	.....	.....	C	.....	.....	.....
2. Hunziker,‡ 1907	58	W	Pelvic recurrence 3 mo. and dyspnea 4½ mo. after surgery	5 mo. after panhysterectomy	UCS + R & C	UCS + R	.....	.....	.....	.....	.....
3. van Akkeren, 1930	60	W	Pelvic recurrence 4 mo. after surgery	11 mo. after panhysterectomy	UCS + C	UCS	.....	UCS	UCS	UCS	UCS §
4. Hartfall (Case 1), 1931	46	W	Dyspnea 14 mo. after surgery	23 mo. after panhysterectomy	UCS + C	.....	.....	UCS (left)	.....	.....	.....
5. Hartfall (Case 2), 1931	54	W	Dyspnea 2 mo. after surgery	6 mo. after panhysterectomy	UCS + C	UCS	UCS	UCS + C	.....	.....	.....
6. Lebowich and Ehrlich,‖ 1941	67	W	Clinical evidence of lung and vertebral metastases with paraplegia	14 mo. after panhysterectomy	UCS + R, C, O, B, & F	UCS + R	UCS + R	UCS + R	UCS + R	UCS + R	.....
7. Pena, 1951	46	W	Dyspnea and left chest pain with pleural effusion 6½ mo. after surgery	8 mo. after panhysterectomy	UCS + C	UCS + C	.....	UCS	.....	.....	UCS ¶
8. Hardy and Moragues,# 1952	29	N	Biopsy of mass protruding from cervical os, with no definitive surgery	Death 23 days after biopsy due to rupture of uterus with peritonitis	UCS + C	.....	.....	+	+	+	.....
9. Kulka and Douglas,** 1953	68	W	Metastatic nodule below urethral meatus 3 mo. after surgery and subsequent vaginal vault recurrence	6 mo. after panhysterectomy	UCS + R	UCS + R	.....	UCS + R	.....	UCS + R	.....
10. Aimauer, 1955	62	W	Pelvic recurrence 3 mo. after surgery	5 mo. after panhysterectomy	UCS + C & R	UCS + C	UCS + C	.....	.....	.....	.....

* The three cases reported by von Franque in 1899, Robertson in 1909, and Shapiro in 1931 are not included in the Table, since no metastases were found at autopsy.
† UCS, undifferentiated cellular sarcoma; R, rhabdomyoblast; C, cartilage; O, osteoid; B, bone; F, fat.
‡ Autopsy limited to the abdomen. Dyspnea highly suggestive of pulmonary metastases.
§ Bone plus tumor thrombus in right femoral vein.
‖ Examination of vertebrae and spinal cord not permitted.
¶ Mediastinum.
# Information regarding histologic composition of metastases not given.
** There was also a separate primary adenocarcinoma of the endocervix. At autopsy metastatic adenocarcinoma to an aortic lymph node was found.

either may be entirely confined to the endometrium or may at most show only superficial invasion of the underlying myometrium. In the majority of the cases the usual period of survival following surgical resection is only 6 to 12 months, and only rarely does the patient survive longer than 18 to 24 months.* In the entire literature only one case, reported by Hartfall[4] (Case 3), had survived five years with no evidence of either local recurrence or distant metastases. A second case, reported by Gamper,[10] had survived four and one-half years, also with no evidence of dissemination.

REPORT OF A CASE

Mrs. M. M., a 62-year-old white woman, was admitted to the United States Naval Hospital, San Diego, Calif., on Dec. 22, 1952, complaining of a watery vaginal discharge for seven weeks, which had become bloody in the last two weeks. There was no weight loss or abdominal pain. The patient had three children, and her last menstrual period occurred at the age of 49 years. Regarding the pertinent past history, the patient had had a known vascular hypertension for several years and had symptoms suggestive of coronary insufficiency.

Physical examination revealed a well-developed obese white woman, who was not acutely ill. Pelvic examination revealed a slight amount of blood oozing from the external os of the cervix, which otherwise was not remarkable. The uterus was not enlarged, and no masses were palpable in the adnexal regions. Except for a blood pressure of 160/110 mm. Hg, the remainder of the examination revealed no significant abnormalities. Vaginal smears stained with the Papanicolaou technique contained no cells indicating a suspicion of malignant transformation. Urinalysis and a complete blood cell count were essentially negative.

On Dec. 23 a cold-knife conization of the cervix and a diagnostic curettage were performed. The histologic description of the curetted tissue will be given with that of the definitive surgical specimen. Chest x-ray on Jan. 1, 1953, revealed no evidence of pulmonary metastases. On Jan. 2 a panhysterectomy, bilateral salpingo-oophorectomy, and appendectomy were performed. The patient made an uneventful postoperative recovery and was discharged from the hospital on Jan. 12.

On April 11 the patient was readmitted because of lower abdominal pain and a sense of pelvic pressure for the previous week. Pelvic examination revealed a small granulating area in the apex of the vaginal vault, on which biopsy was done on April 16. A microscopic diagnosis of "granulation tissue with no evidence of malignancy" was made. Vaginal smears stained with the Papanicolaou technique again contained no cells indicating a suspicion of malignant transformation. A small firm mass was palpable in the right adnexal region. On March 13 chest x-ray and a bone survey revealed no evidence of metastases. The patient was discharged from the hospital on March 22.

The patient was readmitted on May 6 because of continued severe lower abdominal pain. On pelvic examination a hard fixed mass was palpable between the healed vaginal cuff and the upper edge of the healed abdominal incision. Exploratory laparotomy was performed on May 8. A large pelvic mass measuring approximately 18 cm. was found, which was adherent to the surrounding structures. This was hard, irregular and nodular, and reddish-gray. A biopsy specimen was taken, and the incision was closed. The patient's immediate postoperative condition was only fair. On May 10 urinalysis revealed a 2+ albumin and 30 to 40 white blood cells per high-power field. On May 11 the nonprotein nitrogen was 28 mg. and the urea nitrogen, 18 mg. per 100 cc. Roentgen-ray therapy was begun on May 18, with no improvement in the patient's condition. The subsequent clinical course was one of progressive deterioration, and the patient died on June 14.

PATHOLOGIC EXAMINATION OF SURGICAL SPECIMEN

Gross Findings.—The specimen consisted of a previously opened uterus with attached cervix, Fallopian tubes, and ovaries. The symmetrical uterus measured 5.5×6.5×4.8 cm. The serosa was smooth and glistening. A polypoid tumor measuring 3.8×3.5×3.2 cm. completely filled the moderately dilated endometrial cavity (Fig. 1). This was attached to the anterior wall adjacent to the right cornu by a narrow stalk measuring 1.2 cm. in diameter. A portion of the surface was ragged and hyperemic, while the remainder was smooth. The cut surface was moderately firm and gray in the outer portion but was soft and pale tan near its base. The pedicle contained small cystic structures measuring 0.1 to 0.2 cm. The remainder of the endometrial cavity had an extremely thin lining, which was smooth and pale tan. The myometrium averaged only 1 to 1.2 cm. in thickness and contained a few small nodules ranging from 0.6 to 0.8 cm. These had a firm, gray, whorled cut surface. Two separate irregular pieces of tissue, which had broken off from the polypoid tumor, measured 3.8 and 2.8 cm. The first was predominantly gristly and blue-gray (Fig. 2), while the second was soft, shaggy, and reddish-tan.

* References 3, 5, 7, 8, and 9.

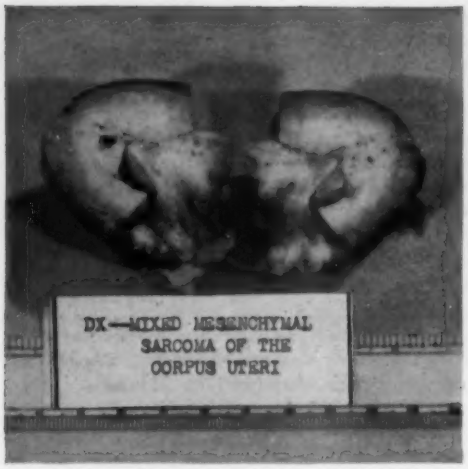


Fig. 1.—Gross photograph of the hemisected corpus uteri showing both halves of the polypoid neoplasm, which is distinctly demarcated from the underlying myometrium.

The cervix measured 3.5×3×2.5 cm., had an irregular gaping external os of 1 cm. diameter, and contained a few cysts of 0.4 cm. average size. The right and left Fallopian tubes measured 6 and 5 cm. in length, respectively, and from 0.4 to 0.6 cm. in diameter. The serosa was smooth and glistening, and the fimbriae at the distal end were well defined. The small right and left ovaries measured 2×1.2×0.8 and 2×1.4×0.9 cm., respectively, and had a solid cut surface, which was firm and gray-white. Also received was an appendix measuring 6 cm. in length and 0.3 cm. in diameter, which on section was a solid cord-like structure.

Microscopic Findings.—Sections through the entire length of the polypoid neoplasm showed it to be highly cellular and composed predominantly of anaplastic mesenchymal cells (Fig. 3). These were oval to spindle-shaped and contained only scanty amounts of cytoplasm. The large oval nuclei were hyperchromatic, with coarse chromatin granules, and showed considerable variation in size and shape. A few huge black nuclei of bizarre type were scattered throughout. Also present were numerous multinucleated tumor giant cells, as well as numerous mitoses of both typical and atypical type.

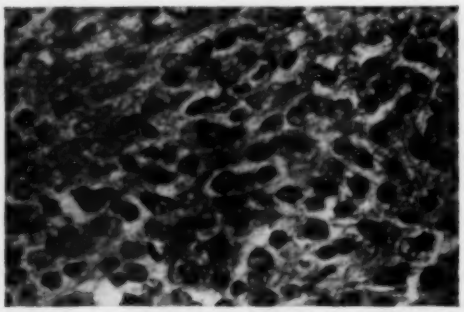
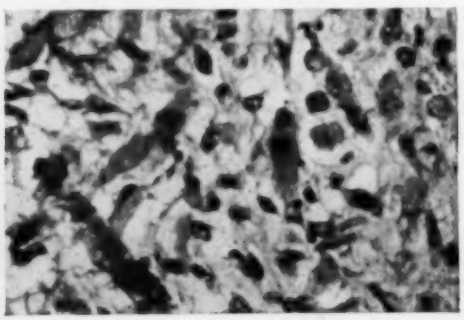


Fig. 3.—High-power photomicrograph showing cellular sarcoma of undifferentiated type, in which several mitoses are present. Reduced ⅙ from mag. × 440.

The two heterotopic mesenchymal components consisted of rhabdomyoblasts and "atypical" hyaline cartilage with the morphologic characteristics of chondrosarcoma. The rhabdomyoblasts contained pleomorphic hyperchromatic nuclei and usually abundant amounts of pink cytoplasm, which took up a brick-red color with Masson's trichrome stain. The smaller ones were round to oval, while the larger ones were elongated with either a narrow ribbon-like or a broader strap-like appearance (Fig. 4). Only a rare

Fig. 2.—Gross photograph of a separate piece of smooth lobulated tissue, which consisted predominantly of cartilage.

Fig. 4.—High-power photomicrograph showing several rhabdomyoblasts, which are broad and strap-like. Cross striations are not demonstrable in these particular cells. Reduced ⅙ from mag. × 440.



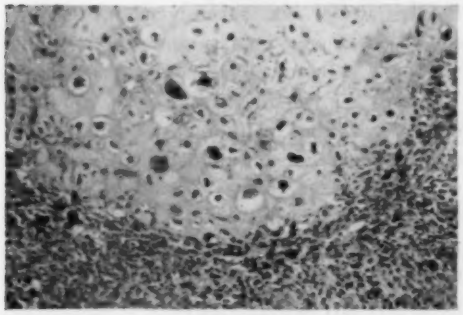


Fig. 5.—Low-power photomicrograph showing anaplastic cellular sarcoma surrounding an island of "atypical" hyaline cartilage with the histologic characteristics of chondrosarcoma. Note the bizarre atypical mitosis. Reduced ⅙ from mag. × 100.

strap-like cell, however, contained well-defined cross striations. An island of cartilage was located near the distal tip of the tumor (Fig. 5). This contained a moderate amount of pale-blue ground substance between the lacunae. The pleomorphic cells within the lacunae contained hyperchromatic nuclei which varied considerably in size and shape. Multinucleated forms were present, as well as a few bizarre mitoses of atypical type. The separate pieces of tissue also were composed of undifferentiated mesenchyme, "chondrosarcoma," and rhabdomyoblasts. This was also the histologic composition of the curetted tissue obtained on Dec. 23, 1952.

Surface ulcerations heavily infiltrated by polymorphonuclear leucocytes as well as foci of hemorrhage and coagulation necrosis were present in the periphery of the tumor. No infiltrating tumor cells were seen in the pedicle, which was composed only of small uniform stromal cells and atrophic endometrial glands. These were lined by a single layer of low columnar cells, and many were dilated and cystic. The line of demarcation between the neoplasm and unaltered endometrium was rather sharp. Near the base, however, a few isolated endometrial glands were completely surrounded by cellular tumor tissue (Fig. 6). No adenocarcinomatous component was present.

A focus of adenomyosis was present in the myometrium beneath the pedicle of the tumor. Neither the glands nor the stroma showed evidence of malignant transformation. The discrete intramural nodules were typical leiomyomas.

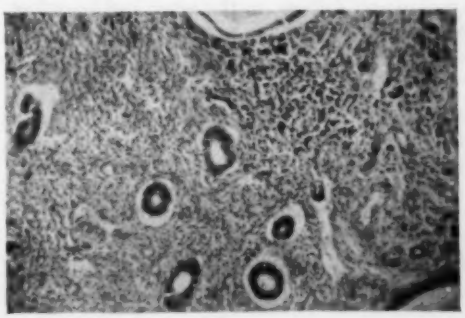
The cervix showed a granulating defect in the endocervical canal, as well as chronic inflammation with Nabothian cysts. The Fallopian tubes and ovaries showed involutional changes and contained no metastatic tumor. The appendix showed fibrous obliteration of the lumen.

Diagnosis: Mixed mesenchymal sarcoma of the corpus uteri, with focal adenomyosis and multiple leiomyomas.

AUTOPSY REPORT

Gross Findings.—The body was that of a well-developed but moderately obese 62-year-old white woman, measuring 5 ft. 4 in. (162 cm.) and weighing 180 lb. (81.6 kg.). No free fluid was present in the peritoneal cavity. The apex of the vaginal vault was well healed. Almost the entire pelvis was filled by a large ill-defined mass measuring approximately 20 cm. This was adherent to the serosal surface of the urinary bladder, rectosigmoid, and loops of small bowel. On section the mass had an irregular cystic center which was surrounded by an intact inner zone of soft, friable, reddish-gray tissue. The firm outer portion contained numerous irregular zones, which were blue-gray and gristly. Numerous nodular tumor implants ranging from 0.2 to 6.0 cm. were scattered throughout most of

Fig. 6.—Low-power photomicrograph showing cellular sarcoma infiltrating non-neoplastic endometrium adjacent to the junction of the neoplasm and its pedicle. Note the bland character of the glands, two of which are cystic. Reduced ⅙ from mag. × 100.



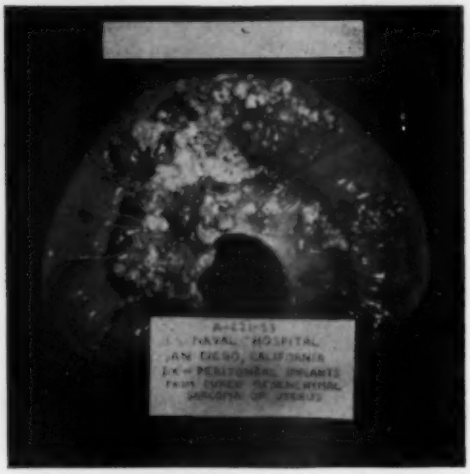


Fig. 7.—Gross photograph of a loop of small bowel showing the tumor implants on both the serosa and the attached mesentery.

the peritoneal cavity. This included the mesentery, serosa of the small bowel (Fig. 7) and colon, omentum, parietal peritoneum, and the leaves of both diaphragms. The omental bursa and the serosa of the liver, spleen, and stomach were not involved. The smaller homogeneous implants were firm and gray-white. The larger ones contained both friable reddish-gray areas and gristly blue-gray areas. Many small bowel loops were plastered together because of the serosal implants. These tumor nodules, however, did not infiltrate the bowel wall, and the mucosa was everywhere intact.

No distant metastases were present in the lungs, heart, liver, spleen, adrenal glands, kidneys, brain, bone, or the abdominal and thoracic lymph nodes.

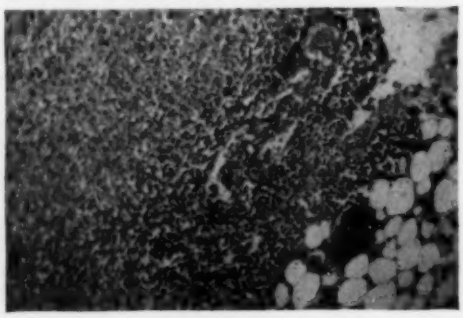
The heart, which weighed 275 gm., contained an area of firm gray-white scar tissue measuring 4.5 cm. in the apex and lower anterior septum. The thickened and tortuous coronary arteries contained numerous yellow intramural plaques with several areas of severe stenosis. The right and left lungs weighed 700 and 650 gm., respectively, and contained a considerable amount of frothy pink fluid. The gall bladder contained several cholesterol calculi.

The left and right kidneys weighed 180 and 150 gm., respectively. The distal portion of the left ureter was severely compressed by the adjacent pelvic mass, while the proximal portion was tortuous and moderately dilated. The renal pelvis and calyces were also moderately dilated. The thinned-out renal parenchyma was rather soft and friable, with poorly defined corticomedullary junction. Numerous irregular yellow streaks with hyperemic margins extended from the medulla to the cortical surface. The right kidney showed no gross change, and the right ureter was patent throughout.

Microscopic Findings. — Numerous sections from the pelvic mass and peritoneal implants were prepared with Masson's trichrome stain, but no rhabdomyoblasts could be found. Otherwise, the recurrent pelvic tumor and peritoneal implants had the same morphologic appearance as the primary neoplasm. There was, however, a striking preponderance of "atypical" hyaline cartilage, which again showed the histologic characteristics of chondrosarcoma. The numerous islands of cartilage were surrounded by cellular zones of undifferentiated mesenchyme (Fig. 8). This was also the histologic composition of the biopsy specimen obtained on May 8, 1953.

Numerous sections of lung were examined, but no arrested tumor emboli were found within the smaller arteries and arterioles. Sections of heart showed a healed myocardial infarct, but no acute myocardial necrosis was seen. The coronary arteries showed marked

Fig. 8.—Low-power photomicrograph showing anaplastic cellular sarcoma surrounding an island of "chondrosarcoma" in the omentum. Reduced ⅓ from mag. × 100.



narrowing of the lumen due to subintimal calcified atheromatous plaques. The kidneys showed slight to moderate arteriolonephrosclerosis and an acute suppurative pyelonephritis of ascending type in the left.

ANALYSIS OF AUTOPSY CASES

In addition to the case presented, 12 other cases of mixed mesenchymal sarcoma of the corpus uteri in which an autopsy was performed have been reported in the literature. Single cases were reported by Wagner † in 1854, von Franque [12] in 1899, Hunziker [13] in 1907, Robertson [14] in 1909, van Akkeren [15] in 1930, and Shapiro [16] in 1931; two cases by Hartfall [4] in 1931 (Cases 1 and 2), and single cases by Lebowich and Ehrlich [5] in 1941, Pena [17] in 1951, Hardy and Moragues [7] in 1952 (Case 2), and Kulka and Douglas [8] in 1952.

In three of these cases no metastases of any type were found. Von Franque's patient, a 58-year-old white woman, died one week after panhysterectomy because of peritonitis. Robertson's patient, a 69-year-old white woman, died of bronchopneumonia two months after admission without definitive surgery. Shapiro's patient, a 52-year-old white woman, also died of bronchopneumonia one day after admission without definitive surgery. The uterine neoplasm in all three cases was composed of cellular sarcoma with rhabdomyoblasts.

In the other 10 cases either a recurrent pelvic mass or distant metastases, or both, were found (Table). As Liebow and Tennant [3] had noted in 1941, the histologic composition of the metastases as compared with that of the primary neoplasm may vary considerably in these cases. With one minor variation (I-*A* and I-*B*), the following categories are essentially the same as those in the classification elaborated by Liebow and Tennant:

† Wagner, E.: Verjauchende Enchondrome des Uterus, Lungenenchondrome, frische Peritonitis, der Gebärmutterkrebs, Leipzig, 1854, p. 129, cited by Williams.[11]

I-A.—All Metastases Are of the Same Histologic Composition as the Primary Tumor

1. In Wagner's case, both the primary tumor and the multiple metastases to the lungs contained cartilage.

2. In Kulka and Douglas' case, the primary tumor was composed of cellular sarcoma with rhabdomyoblasts, as were also the recurrent pelvic mass and the distant metastases to the lungs, pleura, mediastinal lymph nodes, and urethral meatus. This case is also of interest because of a separate primary adenocarcinoma of the endocervix. At autopsy metastatic adenocarcinoma to an aortic lymph node was found.

I-B.—Some Metastases Are of the Same Histologic Composition as the Primary Tumor, but Others Are Composed Only of Undifferentiated Cellular Sarcoma

1. In Hartfall's second case, both the primary tumor and the metastases to the lungs were composed of cellular sarcoma with cartilage. The recurrent pelvic mass and peritoneal implants consisted only of cellular sarcoma.

2. In Pena's case, both the primary tumor and the recurrent pelvic mass were composed of cellular sarcoma with cartilage. The metastases to the lungs and mediastinum consisted only of cellular sarcoma.

II.—The Metastases Contain Some, but Not All, of the Heterotopic Mesenchymal Components Present in the Primary Tumor

1. In Hunziker's case (autopsy limited to the abdomen), the primary tumor was composed of cellular sarcoma with rhabdomyoblasts and cartilage, while the recurrent pelvic mass consisted only of cellular sarcoma with rhabdomyoblasts.

2. In Lebowich and Ehrlich's case, the primary tumor was composed of cellular sarcoma with rhabdomyoblasts, cartilage, osteoid, bone, and fat. The recurrent pelvic mass, peritoneal implants, and the distant metastases to the lungs, liver, and lumbar lymph nodes consisted only of cellular sarcoma with rhabdomyoblasts.

3. In the case presented, the primary tumor was composed of cellular sarcoma with cartilage and rhabdomyoblasts, while the recurrent pelvic mass and peritoneal implants consisted only of cellular sarcoma with cartilage.

III.—The Metastases Consist Only of Undifferentiated Cellular Sarcoma and Contain

None of the Heterotopic Mesenchymal Components Present in the Primary Tumor

1. In van Akkeren's case, the primary tumor was composed of cellular sarcoma with cartilage, while the recurrent pelvic mass and the distant metastases to the lungs, liver, inguinal lymph nodes, second lumbar vertebra, and sacrum consisted only of cellular sarcoma. There was also tumor thrombus in the right femoral vein.

2. In Hartfall's first case, the primary tumor was composed of cellular sarcoma with cartilage, while the distant metastasis to the left lung consisted only of cellular sarcoma.

In Hardy and Moragues' case, the primary tumor consisted of cellular sarcoma containing cartilage. Unfortunately the histologic composition of the distant metastases to the lungs, liver, and retroperitoneal lymph nodes was not given. Also, no photomicrographs of either the primary tumor or the metastases were included.

Although autopsies were not performed, biopsy tissues of metastases were available for histologic study in the cases reported by Glynn and Bell [18] in 1914, Reeb and Oberling [19] in 1929, and Amolsch [9] in 1939 (Case 1). All three cases fall into Category III:

1. In Glynn and Bell's case, a white woman in her 70's, tissue passed spontaneously from the uterine cavity was composed of cellular sarcoma with rhabdomyoblasts. Peritoneal implants obtained at biopsy 16 months later, as well as the remainder of the primary tumor in the uterus resected 13 months later, consisted only of cellular sarcoma.

2. In Reeb and Oberling's case, a 51-year-old white woman, the primary tumor was composed of cellular sarcoma with rhabdomyoblasts. A small pedunculated peritoneal implant on the right broad ligament consisted only of cellular sarcoma. This case is also of interest because of the presence of a separate primary adenocarcinoma in the corpus. This had invaded the "rhabdomyosarcoma" in one area, thus resulting in a "collision tumor." Two months later a metastatic nodule in the lower vagina proved on biopsy to be pure adenocarcinoma, with no trace of sarcoma.

3. In Amolsch's case, a 57-year-old white woman, the primary tumor was composed of cellular sarcoma with rhabdomyoblasts, cartilage, and bone. A biopsy specimen obtained at the time of surgery from numerous small metastatic implants in the peritoneum and omentum consisted only of cellular sarcoma.

HISTOGENESIS

Were it not for the presence of the heterotopic mesenchymal tissues, the mixed mesenchymal sarcoma of the corpus uteri would be indistinguishable from the "pure" endometrial stromal sarcoma. Furthermore, in some of these cases all of the metastases consist only of undifferentiated cellular sarcoma and hence are indistinguishable from the metastases in cases of "pure" endometrial stromal sarcoma. Only a knowledge of the histologic composition of the original primary neoplasm permits distinction between the two. It is my personal opinion that malignant transformation of the endometrial stromal cells, some of which undergo heterotopic differentiation (i. e., malignant metaplasia) to form rhabdomyoblasts and cartilage, also is responsible for the mixed mesenchymal sarcoma.

This concept of aberrant differentiation or metaplasia was first proposed by Pfannenstiel [20] in 1892 and has subsequently been supported by Nicholson [21] and Willis.[22] Recently, essentially the same view regarding the relationship of "heterologous" and "pure" sarcomas of the endometrium was expressed by Ober and Jason,[6] who state that their two cases "are classified, then, as cases of sarcomas of the endometrial stroma because of origin in the endometrium, conformity with some of the properties of the non-neoplastic stroma, and *absence of heterotopic elements* ‡ (notably, cartilage, bone, fat, or muscle)." They were also of the opinion that "rhabdomyosarcoma of the endometrium usually represents a special form of the endometrial stromal sarcoma in which some cells have differentiated as neoplastic muscle cells, a few of them exhibiting striations."

Even such critics of Pfannenstiel's theory as Liebow and Tennant,[3] Lebowich and Ehrlich,§ and Hill and Miller [2] freely concede that malignant metaplasia on the part of the endometrial stroma could account for the presence of cartilage, osteoid, and bone in these neoplasms. Their entire objection to

‡ Italics supplied.

§ References 5 and 23.

this concept of histogenesis is based on the assumption that malignant metaplasia could not possibly account for the presence of rhabdomyoblasts, which must therefore be explained by some other mechanism. These authors have resorted to the time-honored concept of persisting primitive anlage, or "cell rests," in the endometrium as the source for the genesis of these neoplasms.

The original concept of this type was proposed by Wilms,[24] who felt that it was such cell rests associated with the Wolffian duct that gave rise to these tumors. Although they also favor a theory of histogenesis based on "cell rests," Lebowich and Ehrlich,‖ Hill and Miller,[2] and Kulka and Douglas [8] nonetheless feel that Wilms' concept is unsatisfactory because of the fact that the neoplasm develops within the endometrial cavity while the Wolffian duct is located in the outer lateral wall of the uterus. These authors have supported a modified concept proposed by Lahm,[25] who felt that it was the primitive mesoderm from which the Müllerian apparatus developed that gave rise to such "cell rests." Since primitive mesoderm would be "totipotential" (i. e., capable of divergent differentiation into both epithelium and mesenchyme), Lahm's concept had one additional attractive feature. Such primitive anlage would account for the genesis of not only the "heterologous" endometrial sarcoma but also the "heterologous" endometrial carcinosarcoma, which are classified together as the so-called "mixed mesodermal tumor" by many authors.¶

The crux of this theoretical problem of origin hinges on the validity of the assumption that the rhabdomyoblast truly is the *sine qua non* of these tumors and that theories of histogenesis must accordingly take it into account.

I feel that the most significant objection to the above assumption is the rather obvious fact that the rhabdomyoblast is not the common denominator of the "heterologous" endometrial sarcomas. Many of these tumors simply do not contain rhabdomyoblasts. Although it might be contended by some # that the "pure" rhabdomyosarcoma and "pure" chondrosarcoma are two entirely separate neoplasms in which totally different mechanisms of histogenesis are involved, it is equally apparent that these merely represent minor variants of the same neoplasm. Several such tumors described in the literature actually contain both rhabdomyoblasts and cartilage.* Furthermore, the metastases in some cases consist only of cellular sarcoma without rhabdomyoblasts, even though these were present in the primary neoplasm (the cases reported by Glynn and Bell, Reeb and Oberling, Amolsch, and the case presented). Since the rhabdomyoblast is not a consistent feature either in the primary neoplasms or in the metastases, it certainly constitutes a poor foundation on which to erect a reasonable and logical theory of histogenesis.

An additional logical objection to "cell rests" as the explanation for the origin of rhabdomyoblasts has been expressed by Willis [22]:

> The supposition that undifferentiated "rests" of developmentally heterotopic tissues may remain in the endometrium for the whole of a woman's reproductive life, surviving multiple pregnancies, and eventually producing a mixed embryonic tumour at the age of 50 or 60 or 70 is absurd. . . . Muscular tissue, then, especially striated muscle, is the only ingredient of mixed tumours that need excite any wonder or call for any special hypothesis regarding histogenesis. The development of muscle by aberrant differentiation in a plastic mesenchymal tumour tissue which has displayed its plasticity by producing also cartilage, bone, and adipose tissue, is a much more probable event than the life-long retention of rests of embryonic tissue of multiple kinds.

RELATIONSHIP OF MIXED MESENCHYMAL SARCOMA AND "HETEROLOGOUS" CARCINOSARCOMA OF THE CORPUS UTERI

The lack of an intrinsic adenocarcinomatous component is the significant characteristic which distinguishes the mixed mesenchymal, or "heterologous" endometrial,

‖ References 5 and 23.

¶ References 3, 5, 7, 9, 23, and 26 through 30.

References 5 and 23.

* References 5, 7, 9, 10, 13, 23, 28, and 31.

sarcoma from the "heterologous" carcinosarcoma (i. e., carcinosarcoma with heterotopic mesenchymal components). I feel that there is a logical explanation for the genesis of the latter neoplasm, which is completely in accord with that for the genesis of the former tumor.

I consider the following cases in the literature to be highly significant because of the presence of two separate neoplasms in the corpus uteri. In the case reported by Ehrlich [23] in 1942, one was an adenocarcinoma and the other a "heterologous" endometrial sarcoma containing both rhabdomyoblasts and cartilage. In the cases reported by Reeb and Oberling [19] in 1929 and Poole [32] in 1943, one was an adenocarcinoma and the other a rhabdomyosarcoma. In the case reported by Goodfriend and Lapan [33] in 1950 (unfortunately and erroneously termed a "carcinosarcoma"), one was an adenocarcinoma and the other a "pure" endometrial stromal sarcoma without heterotopic mesenchymal components. Although they were separate and distinct, "collision" of the tumors had occurred in the latter three cases. In one additional case, reported by Hall and Nelms [34] in 1953, one was a "pure" adenocarcinoma and the other a carcinosarcoma of "heterologous" type, which was composed of cellular sarcoma, cartilage, rhabdomyoblasts, and an intrinsic adenocarcinomatous component. There was no "collision" between the two tumors in the cases reported by Ehrlich and by Hall and Nelms.

In view of such cases, it is apparent that the endometrial glands in one area and the endometrial stroma in another area can undergo malignant transformation simultaneously with the consequent formation of a "pure" adenocarcinoma and either a "heterologous" or a "homologous" sarcoma, respectively. Therefore it seems quite reasonable that both endometrial glands and stroma in the same area may simultaneously undergo malignant transformation, with the consequent formation of a single neoplasm containing intrinsic components from both sources. The type of carcinosarcoma (i. e., "heterologous" or "homologous") would merely depend on the presence or absence, respectively, of heterotopic mesenchymal components due to malignant metaplasia on the part of the neoplastic stromal cells. That such tumors are a single neoplastic unit is established by the presence of both carcinomatous and sarcomatous elements in the metastases as well as in the primary tumor in Nicholson's case,[21] Hill and Miller's first two cases,[2] and Case 6 reported by Wilson and co-workers.[29]

Recently, Sternberg, Clark, and Smith [35] have expressed their skepticism regarding all variations of the basic "cell rest" concept. Nonetheless, they, too, have attempted to explain the genesis of both "heterologous" endometrial sarcoma and "heterologous" carcinosarcoma by means of a single mechanism. According to their concept both neoplasms "arise from the specific stroma that underlies the epithelium of much of the genital tract, of which the most conspicuous example is the endometrial stroma. Such stroma, under the appropriate neoplastic stimulus, can apparently give rise to all of the histological elements seen in the tumors."

Thus, according to these authors, the endometrial stroma is responsible not only for the mesenchymal but also for the carcinomatous component of the "heterologous" carcinosarcoma, while the preexisting endometrial glands play no role whatsoever in the formation of the intrinsic epithelial component.

Regarding the genesis of the mixed mesenchymal sarcoma alone, I am in full accord with the concept proposed by Sternberg, Clark, and Smith. I do not, however, believe that this concept is necessary to account for the genesis of the "heterologous" carcinosarcoma. No one seriously maintains that malignant transformation of the endometrial stroma is responsible for the development of the "pure" adenocarcinoma of the corpus uteri. Such being the case, there is no logical reason for the endometrial stroma to be utilized in accounting for the presence of the intrinsic adenocarcinomatous component in the "heterologous" carcinosarcoma.

SUMMARY

A case of mixed mesenchymal sarcoma of the corpus uteri in a 62-year-old white woman is reported. The primary neoplasm was composed of undifferentiated cellular sarcoma containing rhabdomyoblasts and cartilage. At autopsy, five months after panhysterectomy, a recurrent pelvic tumor and widespread peritoneal implants consisting only of cellular sarcoma with cartilage were found.

Twelve similar cases in which an autopsy was performed are recorded in the literature. In three cases there was no dissemination of any type. Including the case presented, the sites of dissemination in the other 10 cases are as follows: (1) in 2 there was only a recurrent pelvic tumor; (2) in 3 there were only distant metastases, and (3) in 5 there were both distant metastases and a recurrent pelvic tumor. In three of the cases with a local pelvic recurrence widespread peritoneal implants were also present.

These cases show the following variations between the histologic composition of the metastases and that of the primary neoplasm: (1) in two cases both the primary tumor and the metastases had the same histologic composition; (2) in two cases only some of the metastases had the same histologic composition as the primary tumor, while others were composed only of undifferentiated cellular sarcoma; (3) in three cases the metastases contained some, but not all, of the heterotopic mesenchymal components present in the primary tumor; (4) in two cases the metastases consisted only of undifferentiated cellular sarcoma. This information was not available in one case.

The mixed mesenchymal sarcoma, or "heterologous" endometrial sarcoma, is considered to be due to malignant transformation of the endometrial stromal cells, with aberrant differentiation (i. e., malignant metaplasia) accounting for the heterotopic mesenchymal components.

The relationship of the "heterologous" endometrial sarcoma and the "heterologous" carcinosarcoma of the corpus uteri is discussed, and a concept of histogenesis for the latter neoplasm is also presented.

Comdr. Charles Hascall (MC), U. S. N., of the Pathology Service, United States Naval Hospital, San Diego, Calif., assisted in taking the photomicrographs; Donald E. Reeves (HM_1), U. S. N., of the Pathology Service, prepared the Masson trichrome stains, and William Von Allmen (HM_2), U. S. N. R., of the Medical Photography Service, prepared the gross photographs and developed the photomicrographs.

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Schistosoma Haematobium Infestation and Hepatic Disease in Man

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The relationship of many parasitic diseases to periportal fibrosis, cirrhosis of the liver, and primary hepatic cancer is still obscure, especially in tropical centers, where such lesions of the liver are widespread. This uncertainty is also true for schistosomiasis, but the belief is now gaining ground that Schistosoma may not be a significant factor in causing diffuse liver disease.* On the other hand, early workers such as Fairley [4] and Dew [5] were certain that significant liver damage resulted from infestation by both Schistosoma mansoni and S. haematobium due to toxins released by the parasite, and Girges refers to a hepatic type of schistosomiasis in Egypt.[6] More recently, Jaffé,[7] in Venezuela, has expressed the opinion that S. mansoni definitely causes liver damage.

In view of this diversity of opinion, it would appear advisable to review the relationship of schistosomiasis to liver disease in man, especially in regard to degree of infestation. For this reason we consider it worth while to publish our material from an area of moderate infestation by S. haematobium and to a lesser extent by S. mansoni.

As S. japonicum is not seen in Africa, no mention is made of this parasite.

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* References 1 through 3.

REVIEW OF LIVER LESIONS ATTRIBUTED TO SCHISTOSOMIASIS

According to various authors the following hepatic lesions have been attributed to schistosome infestation.

(*a*) *Bilharzial Tubercle.*—The characteristics of the Bilharzial tubercle are well known and have been described in detail by Fairley.[4] It is found frequently in the liver at necropsy in both S. haematobium and S. mansoni infestation by pathologists working among subjects in whom schistosomiasis is widespread. Scattered tubercles can be observed in the liver without either periportal fibrosis or cirrhosis. In the late stages, ova may be absent and only a small fibrous scar remains to show the site of the tubercle. Such tubercles and fibrous scars are not infrequently seen in livers in South Africa (Figs. 1 and 2).

(*b*) *"Pipe-Stem" Cirrhosis.*—This is a rare lesion and in Egypt has been described as forming only 1% to 2% of all cases of hepatic cirrhosis.† In Johannesburg, this type of cirrhosis is so infrequent as to be of no significance, and in over 3000 necropsies we have only seen two such livers. The pathological features are distinct from those of classical Laennec's or portal cirrhosis (atrophic cirrhosis). On section, thick cuffs of fibrous tissue are found in the region of the larger veins. In the fibrous tissue numerous Bilharzial ova can usually be observed, the number of ova probably depending on the duration of the lesion (Figs. 3 and 4). Dew [5] states that this type of cirrhosis is more frequent in S. mansoni than S. haematobium infection. Nodular hyperplasia and distortion of the liver architecture are not a feature.

† Symmers, D. St. C. (1904), cited by Gelfand.[2]

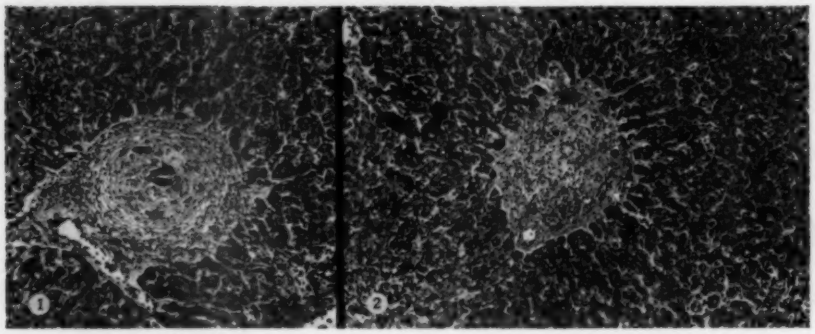


Fig. 1.—Medium-power photomicrograph of typical Bilharzial tubercle; reduced ⅓ from mag. × 150.

Fig. 2.—Fibrous scar in same liver as Figure 1. Several Bilharzial tubercles were present in this liver, also areas of focal fibrosis in which no ova could be demonstrated. It is probable that this lesion represents a late stage of the tubercle. Reduced ⅓ from mag. × 150.

(*c*) *Periportal Fibrosis and* (*d*) *Portal Cirrhosis.*—There is no certainty of opinion as to whether or not schistosomiasis may produce periportal fibrosis and portal cirrhosis. Gelfand [2] compared the incidence of cirrhosis in different parts of Africa and concluded that schistosomiasis does not cause portal cirrhosis. On the other hand, Fairley and Dew believed that periportal fibrosis in the liver can definitely result from schistosomiasis in man.‡

In animals, focal fibrosis and annular cirrhosis have been produced by experimental infestation with S. mansoni, S. japonicum, and S. haematobium by Meleney and his coworkers,§ the pathological picture varying to a certain extent according to the species used. Fairley [4] described periportal fibrosis in monkeys after schistosome infestation. More recently, however, Bersohn and Lurie [10] were unable to find any periportal fibrosis in monkeys infected with S. bovis, even after 25 weeks.

Few authors, however, in either animal or human studies have distinguished between the results of mild and heavy infestation, and in most animal studies infestation has been heavy.

‡ References 4 and 5.

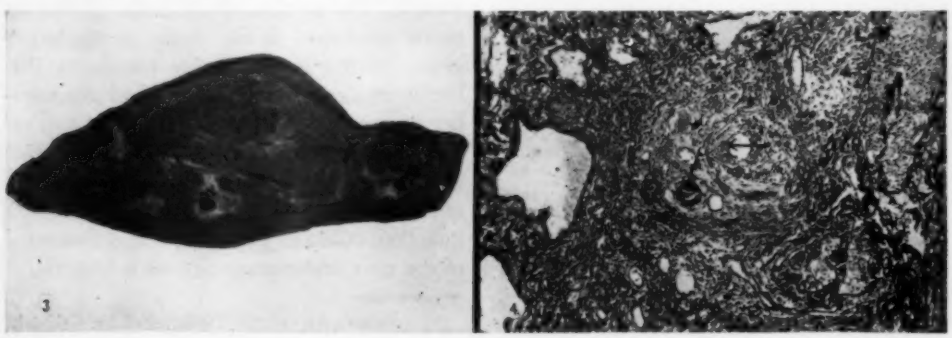
§ References 8 and 9.

MATERIAL AND METHODS

Our material was obtained from native Bantu subjects who had died at Baragwanath Non-European Hospital and who had lived in the urban area of Johannesburg for varying periods. In

Fig. 3.—Typical "pipe-stem" cirrhosis in case with mixed S. mansoni and S. haematobium infestation.

Fig. 4.—High-power view of portal tract in the same liver as Figure 3. The arrows show scattered ova. Reduced ⅓ from mag. × 120.



Johannesburg infection with schistosomiasis is very unlikely, and it is probable that the majority of infections were acquired outside the city. The factor, therefore, of recent repeated multiple infection is unlikely to be of major importance in this series. It is not known how many patients in this series of either sex were born in the Johannesburg area or how many later migrated there from a rural area.

Apart from one subject with "pipe-stem" cirrhosis, who died of ruptured esophageal varices, the finding of schistosome ova was in each case incidental and did not cause significant structural damage in any organ. The infestation in this series can accordingly be regarded as mild.

A total of 235 unselected necropsies were examined. These were consecutive, except when not possible due to vacations and periods of staffing difficulties. None of these factors should cause any degree of selectivity in relation to parasitic infestation. As there were only 7 cases of primary cancer of the liver in this series, 8 more consecutive cases of hepatoma were selected, making a total of 15 primary hepatic cancers.

Examination for Schistosome Ova.—The rectum and bladder in each necropsy, including the genital organs in the female cases, were digested in 10% sodium hydroxide at 60 C overnight, and the digest was examined microscopically for the presence of ova. This method, which was also used by Gelfand,[2] was found to be necessary, as in our experience it is not possible to exclude the presence of mild schistosome infestation at necropsy by naked eye examination alone, nor did facilities permit the examination of multiple sections from the rectum or bladder.

In addition, in 94 consecutive subjects 500 to 600 gm. of liver tissue were also digested and examined for ova. Liver examination was not done in the entire series, as we found infestation of the liver rare in the absence of involvement of the bladder and rectum. Gelfand,‖ in a detailed study in which many different viscera were separately digested, had a similar experience and only found infestation in the liver in the absence of the rectum and bladder infestation in 1% to 2% of infected cases. We therefore feel that the failure to digest the liver in all cases will not significantly affect our results, which provide a reasonably accurate estimate for the incidence of schistosome infestation at necropsy.

‖ Gelfand and Ross,[11] and Gelfand, private communication.

GRADING OF THE LIVER LESIONS

Elsewhere we have made a distinction between the two major types of liver disease in the South African Bantu associated with fibrosis as follows.¶

(*a*) A fine monolobular periportal fibrosis commonly called "tropical or nutritional cirrhosis," which is seldom fatal and is usually an incidental observation at necropsy. Originally, in common with some authors we have called these livers cirrhotic, but as hyperplasia and distortion of the hepatic architecture is not a significant feature, even in the annular phase, we have not used this term in the present study in accord with current American practice.[14] In Johannesburg the majority of such livers are associated with a well-marked siderosis [12] (Fig. 5).

(*b*) A coarse portal cirrhosis (atrophic cirrhosis) in which parenchymal cell hyperplasia, venous anastomoses, and distortion of the liver architecture are marked. We believe that the majority of such livers in the Johannesburg Bantu are examples of postnecrotic scarring (Fig. 6).

For the purpose of this paper, therefore, the livers in our series were classified as follows:

Group 1: Livers with essentially normal histology and without significant periportal fibrosis.

Group 2: Livers with a diffuse increase in periportal fibrous tissue but without significant hyperplasia or distortion of architecture (Fig. 5).

Group 3: Livers with a coarse multilobular cirrhosis in which distortion of architecture is prominent. This group corresponds to the classical atrophic or portal cirrhosis (Fig. 6).

This classification of livers was carried out before the results of the ova examination were available. Focal Bilharzial tubercles and isolated fibrous scars in the liver were ignored in this grading.

¶ References 12 and 13.

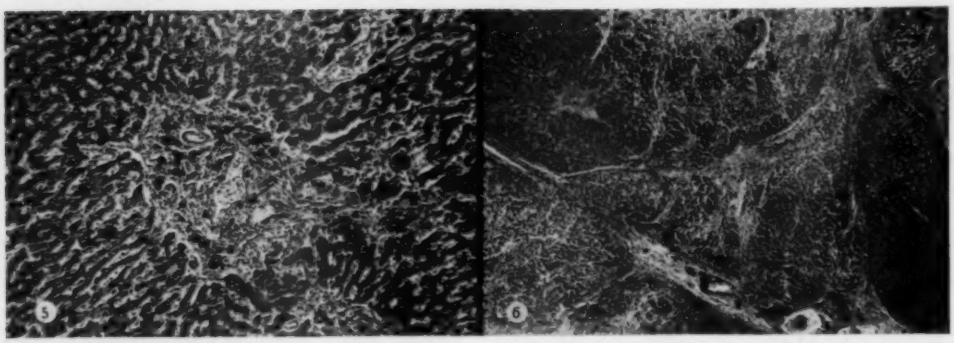


Fig. 5.—Increased fibrosis on the portal tracts associated with hemosiderin deposition; reduced ⅓ from mag. × 120.

Fig. 6.—Portal cirrhosis, probably of postnecrotic origin in male subject of 15 years; reduced ⅓ from mag. × 30.

TABLE 1.—*Relationship of Schistosomiasis to Liver Disease in Male Subjects*

	Degree of Liver Damage (Excluding Primary Liver Cancer)									
	1. Normal Architecture *		2. Periportal Fibrosis * †		3. Portal Cirrhosis *		4. Acute Necrosis *		5. Total *	
Age Group	Ova Absent	Ova Present	Ova Absent	Ova Present	Ova Absent	Ova Present	Ova Absent	Ova Present	Ova Absent	Ova Present
0-14 yr.....	9	1	2	0	0	1	0	0	11	2
15-34 yr.....	17	3 (1)	7	3	0	2 (2)‡	2	0	26	8 (3)
35-54 yr.....	23	8 (1)	16	7 (1)	2	0	0	2	41	17 (2)
55 yr. and over	3	3	14	3	1	0	0	0	18	6
	52	15 (2)	39	13 (1)	3	3 (2)	2	2	96	33 (5)

* The figures in parentheses indicate the number of cases in which both S. mansoni and S. haematobium infestation was present.

† Two cases of mixed central and portal fibrosis due to chronic venous congestion are included in Column 2, one of which was associated with S. haematobium.

‡ Includes one case of "pipe-stem" cirrhosis.

TABLE 2.—*Relationship of Schistosomiasis to Liver Disease in Female Subjects*

	Degree of Liver Damage (Excluding Primary Liver Cancer)									
	1. Normal Architecture *		2. Periportal Fibrosis *		3. Portal Cirrhosis *		4. Acute Necrosis *		5. Total *	
Age Group	Ova Absent	Ova Present	Ova Absent	Ova Present	Ova Absent	Ova Present	Ova Absent	Ova Present	Ova Absent	Ova Present
0-14 yr.....	18	0	1	0	0	0	1	0	20	0
15-34 yr.....	21	0	3	1 (1)	0	0	1	0	25	1 (1)
35-54 yr.....	19	0	14	0	2	0	1	0	36	0
55 yr. and over	6	0	7	1	2	0	0	0	15	1
	64	0	25	2 (1)	4	0	3	0	96	2 (1)

* The figures in parentheses refer to those cases with both S. mansoni and S. haematobium infestation.

TABLE 3.—*Relationship of Schistosomiasis to Primary Carcinoma of the Liver*

	Males		Females		Total	
Age Group	Ova Absent	Ova Present	Ova Absent	Ova Present	Ova Absent	Ova Present
15-34 yr.....	3	1	1	0	4	1
35-54 yr.....	6	2	0	0	5	2
55 yr. and over.....	0	1	1	0	1	1
Totals.....	9	4	2	0	10	4

RESULTS

The results of this study are presented in Tables 1 to 3. Among the 130 male subjects without liver cancer, there was 1 case in which S. mansoni ova alone were recovered, and this has been excluded. Among the remaining 129 subjects, there were 28 cases (22%) in which S. haematobium ova alone were found and 5 (4%) in which both S. mansoni and S. haematobium ova were present. There were two cases (2.1%) among female subjects in which schistosome ova were found. We are unable to explain this marked sexual difference, but it may reflect the habits of the two sexes in relation to bathing, washing, etc.

It will be seen from these Tables that there is no significant correlation between the presence of schistosome ova in the pelvic viscera and periportal fibrosis in the liver, and this observation is confirmed by statistical analysis. In males excluding cases under 15 years of age, schistosome ova were demonstrated in 32.6% of essentially normal livers and 33% of livers with periportal fibrosis.

The number of cases of hepatic necrosis and portal cirrhosis in each sex is too small to permit any conclusions. But for the purpose of argument, if one accepts the hypothesis that the pathogenesis of portal cirrhosis and necrosis is not essentially different for each sex in the Johannesburg area, it would appear that schistosomiasis is not a significant etiological factor in causing portal cirrhosis and necrosis. Of 94 cases in which both liver and pelvic viscera were examined, ova were found in 12, but in only 2 of these were they also demonstrated in the liver.

Schistosome ova (all S. haematobium) were found in 4 (27%) of 15 cases of primary hepatic carcinoma, and in 2 of these ova were demonstrated in the liver (Table 3).

The mean weight of livers from male subjects with schistosomiasis was 1666 gm., and in livers from male subjects without schistosomiasis, 1688 gm. The corresponding figures for the spleen were 263 gm. and 233 gm., respectively.

COMMENT

It would appear from the results obtained in this study that mild schistosome infestation is not a significant factor in giving rise to periportal fibrosis, portal cirrhosis, or hepatic enlargement. It is possible, of course, that in some cases a preexisting schistosome infection may have been present which has undergone self-healing with loss of ova. In a study of this nature, exclusion of such an explanation is not possible. Further, that Bilharzial ova can disappear is suggested by experimental work on animals.# On the other hand, we feel that if the correlation of schistosomiasis with diffuse liver fibrosis and cirrhosis is of considerable importance, ova should be very much more frequently found in cases with portal fibrosis and cirrhosis than in apparently normal livers, nor would the above hypothesis explain the sex differences. For these reasons, we consider ourselves justified in believing that periportal fibrosis and portal cirrhosis as found in the Johannesburg area are not significantly related to mild infestation by S. haematobium. This does not exclude the possibility that the results of heavy repeated infections in man may lead to liver damage of the degree seen experimentally.*

The number of primary hepatic carcinomas in this series is small, but our observations again suggest that schistosomiasis is not a major factor in causing the high incidence of primary liver cancer in Johannesburg. Our results are in accordance with those of Afifi [15] in Egypt, where despite widespread schistosome infestation (90%) liver cancer appears to be uncommon. The rate of infestation in this series is much lower than that reported from Rhodesia by Gelfand, who found ova in 80% of necropsies.[2] It is much higher, however, than the rate of 1% of necropsies previously cited from the Johannesburg area when the digestive technique was not used, thus emphasizing the value of this method.[16]

SUMMARY

In a series of 243 necropsies in Johannesburg, a region of mild schistosome infestation, the pelvic organs were digested in a strong alkali and examined for schistosome ova. The presence or absence of ova was cor-

References 8 through 10.

* References 8 and 9.

related with the state of the liver. No significant correlation was demonstrable between liver fibrosis, cirrhosis, and primary hepatic cancer and the presence of schistosomiasis.

Mr. E. H. Hollingham and Mrs. V. Traill, of the Bilharzia Natural History Unit of the Council of Industrial and Scientific Research, gave technical assistance, and Mr. M. Ulrich supplied the photomicrographs.

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Fluoride and Azide Effects o.. in Vitro Metabolism of Breast Cancer

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It is generally recognized that breast cancer cases vary in their response to hormone therapy, castration, and adrenalectomy. While an appreciable percentage of cases 60 years of age and older will respond to estrogenic therapy, premenopausal cases are more adequately treated with antiestrogenic measures. In addition, a large per cent of cases, regardless of age, fail to respond to any of the available modalities of hormonal imbalance procedures.

Such variations in the response of the tumor to manipulation of the hormonal milieu of the host would suggest that there might be corresponding differences in the metabolism among individual breast cancers. The present report is a presentation of data which are highly suggestive of a relationship between the hormonal status of the host and the metabolic activity of breast cancer tissue as determined by in vitro measurements. These data represent a phase of a continuing study of the in vitro dehydrogenase activity of cancer tissues with particular reference to the effects of enzyme inhibitors.

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This study was aided by grants from the Leukemia Research Foundation, Inc., and the Schering Corporation.

MATERIAL AND METHODS

Measurements were made of the in vitro dehydrogenase activity of tissue slices from 112 unselected cases of human breast carcinoma. The tumor tissues were obtained from the operating room of the Flower and Fifth Avenue Hospitals, and the analyses were initiated within 10 minutes of removal. Tissue slices were prepared, and the in vitro endogenous dehydrogenase activity was evaluated in terms of the micrograms of 2,3,5-triphenyltetrazolium chloride (TTC) reduced per milligram of tissue according to techniques previously described.[1] Similar measurements were also made in the presence of fluoride and azide, and the difference in per cent from the endogenous value was calculated. The details of these latter procedures have also been presented previously.[2]

The data obtained were then evaluated in accordance with the age of the patients. For this purpose, the total series was divided into three groups—young (<45 years of age), middle (45 to 59 years of age), and old (>60 years of age)—and a comparison was made of the various metabolic data among the three individual groups.

RESULTS

Endogenous Dehydrogenase Activity.—Measurements were made of the micrograms of TTC reduced/mg of tissue from 112 cases of breast carcinoma. While the values obtained varied from 3.0γ/mg to 11.0γ/mg, almost 80% of the cases yielded values between 5.0γ/mg to 8.9γ/mg, inclusive. The

TABLE 1.—*Distribution of Intensity of In Vitro Endogenous Dehydrogenase Activity of Human Breast Cancer Slices in Relation to Age of Host*

Age, Yr.	No. of Cases	γ TTC Reduced Per Mg. Tissue				
		3.0-4.9	5.0-6.9	7.0-8.9	9.0-10.9	11.0
45	29	7%	10%	50%	31%	3%
45-59	50	30%	24%	26%	14%	6%
60	33	6%	48%	24%	18%	3%
Total	112	17%	28%	31%	20%	4%

TABLE 2.—*Percent Incidence of Fluoride Effects on Dehydrogenase Activity of Breast Cancer Tissue Slices*

Age, Yr.	No. of Cases	>21	20 to 6	5 to −10	−11 to −25	>−26
45	24	50%	25%	4%	4%	8%
45-59	45	38%	20%	22%	16%	4%
60	32	27%	13%	30%	12%	15%
Total	101	45%	19%	21%	12%	9%

TABLE 3.—*Percent Incidence of Azide Effects on Dehydrogenase Activity of Breast Cancer Tissue Slices*

Age, Yr.	No. of Cases	21	20 to 6	5 to −10	−11 to −25	−26
45	18	22%	6%	22%	28%	22%
45-59	27	20%	20%	15%	20%	32%
60	23	4%	9%	13%	31%	43%
Total	68	15%	12%	15%	25%	33%

distribution of values found in the entire series and in the groups divided according to the age of the patients is indicated in Table 1. It will be noted that the distribution curves in the young and old groups are quite similar to one another and to the total series.

Fluoride Effect.—The effect of fluoride on the in vitro dehydrogenase activity was determined in tissue slices of 100 cases of breast carcinoma. The effects varied from inhibitions of more than 50% to increments of TTC reduction greater than 50%. In Table 2 we have indicated the relative frequency of the varying effects of fluoride as found in the total series and in relation to the age group of the patients. It will be noted that 20 of the 24 tissues from patients less than 45 years of age were stimulated more than 5% by fluoride. More than half of the cases were stimulated more than 20%. In contrast, in the old group only 28% of the tissue slices were stimulated to the latter degree, while approximately one-fourth of the cases were inhibited at least 11%. The data obtained in the study of the middle group revealed a decided similarity to the distribution found in the total series.

A comparison of the incidence per cent of the various fluoride effects as found in the young and the old series according to the χ^2 method, employing 4 degrees of freedom, yielded a value of 19.27, which corresponds to a P value of <0.01.

Azide Effect.—The effect of sodium azide on the in vitro dehydrogenase activity was determined in tissue slices from 67 cases. In Table 3 we have indicated the incidence of the various degrees of stimulation and inhibition observed in the total series and in accordance with the age group of the patients. These data indicated that in the old group stimulation greater than 29% was found in only 1 of the 24 cases (4%), whereas in the young group 4 of 18 cases (22%) showed such stimulation. On the other hand, inhibition greater than 10% was found in more than 70% of the old group but in only 50% of the young group. The probability that these differences were due to chance is less than 1 in 100 (χ^2 18.10, $P<0.01$).

Hormonally Treated Cases.—In addition to the series of cases described above, tissues were obtained from five patients who had been treated with testosterone for at least one week prior to removal of a skin metastasis from their breast carcinomas. We also obtained breast cancer tissue from one patient who had been receiving estrogen therapy for several years for treatment of a

TABLE 4.—*Dehydrogenase Activity of Breast Cancer Slices from Patients Treated with Hormone Therapy*

Case	Age, Yr.	Hormone Therapy	Endogenous	Fluoride Effect	Azide Effect
1134-54	34	Testosterone	9.01	−10%	.....
3273-53	42	Testosterone and castration	9.22	−30%	.....
4557-52	48	Testosterone	4.15	−54%	.....
4625-53	47	Testosterone and castration	10.53	−48%	−57%
4251-54	55	Testosterone and castration	9.73	−25%	.....
2838-53	56	Estrogens	10.79	+70%	+31%

TABLE 5.—*Percent Incidence of Fluoride and Azide Groups in Relation to Age of Patients*

Age, Yr.	No. of Cases	Fluoride and Azide Group: Pos.-Pos.*	Neg.-Neg.†	Pos.-Neg.‡
45	18	22%	6%	72%
45-59	27	22%	33%	45%
60	23	4%	61%	35%
Total	68	16%	35%	49%

* Positive-positive, both fluoride and azide effect, >6% stimulation.
† Negative-negative, both fluoride and azide effect, <6% stimulation or actual inhibition.
‡ Positive-negative, positive effect by one reagent, negative by the other.

menopausal syndrome. Pertinent data obtained in regard to the in vitro dehydrogenase activity of these cases are presented in Table 4. It will be noted that all five cases who had received testosterone therapy showed fluoride inhibition of the dehydrogenase activity of their tumor tissue slices. All of the cases were less than 56 years of age, and all had been actively menstruating before the antiestrogenic therapy. It should also be mentioned that a pretreatment study had been made in one case (1134-54) at the time of the original mastectomy, at which time the comparative data were the following: endogenous value, 8.45γ TTC/mg; fluoride effect, +69%; azide effect, +9%.

In the case of the menopausal patient receiving estrogen therapy, the tissue metabolic studies revealed a marked stimulation by both fluoride and azide, 70% and 31%, respectively.

COMMENT

The above data strongly suggest that a systemic influence is exerted upon the metabolic activity of breast cancer tissue in an appreciable percentage of cases. Thus, the dehydrogenase activity of breast cancer tissue slices from patients less than 45 years of age tended to be stimulated by fluoride and was infrequently inhibited by this agent. In contrast, analogous studies performed on breast cancer tissues from patients 60 years or older gave evidence of inhibition in more than 40% of the cases. In addition, the studies with azide also showed a difference between the reactions of the tissue slices from the young and from the old group. That these differences were related to the difference in hormonal status in the two groups is further suggested by the observations on the series of cases receiving hormone therapy before biopsy.

In order further to evaluate the apparent correlation between the age groups and the metabolic activity of the tissue slices, we analyzed the relationship between age and both fluoride and azide effects on the individual cases. In Table 5 we have indicated the incidence of both fluoride and azide effects as found in the different age groups. From these data it is clear that stimulation of the metabolic activity of the slices by both fluoride and azide was uncommon in the older age group. It might be stated that if both fluoride and azide stimulated the dehydrogenase activity of a breast cancer tissue slice, it would be unlikely that the tumor host was 60 years of age or older. In contrast, if both of these agents inhibited the dehydrogenase activity, it would be unlikely that the host was less than 45 years of age.

In short, the cases of the young group tend to fall into the positive-fluoride positive-azide type of response much more frequently than do the old-age cases (22% *vs.* 4%). On the other hand, the old-age cases occurred in the negative-fluoride negative-azide division much more frequently than did the young cases (61% *vs.* 6%). These differences between the young and the old groups are significant at less than the 1% level (χ^2 44.22, $P<0.01$). Further indication of this difference is found in regard to the age incidence of the cases comprising the different fluoride-azide groups. These data are indicated in Table 6. It will be noted from this tabulation that the metabolic criteria provided by the

TABLE 6.—*Percent Incidence of Age Groups in Relation to Fluoride and Azide Effects*

Fluoride-Azide Group	No. of Cases	Age Groups: 45 Yr.	45-59 Yr.	60 Yr.
Pos.-pos.	11	40%	60%	10%
Neg.-neg.	24	4%	37%	58%
Pos.-neg.	33	39%	30%	24%
Total	68	27%	40%	34%

fluoride and the azide effects gave evidence of three types of response related to the age of the host: (*a*) positive-positive group, characterized by younger individuals; (*b*) negative-negative group, composed predominantly of older patients, and (*c*) positive-negative group, no age predilection apparent. Such groupings based on the metabolic activity are suggestive of the clinical groupings of hormonally dependent and independent cases. Studies are in progress to assess the applicability of these metabolic data to the problem of hormone therapy in breast cancer cases.

SUMMARY

Measurements were made of the in vitro dehydrogenase activity of human breast cancer tissue slices in the presence and absence of fluoride and azide. These data were then evaluated in relation to the age of the patients. It was found that stimulation of the dehydrogenase activity by both fluoride and azide was infrequent in breast cancer tissues from patients 60 years of age or older. In contrast, inhibition by both fluoride and azide was uncommon in cancer tissues from patients less than 45 years of age.

The findings were discussed in relation to the clinical response of human breast cancer to hormone imbalance therapy.

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News and Comment

ANNOUNCEMENTS

Applications for Grants in Cancer Research.—Acting for the American Cancer Society, the Committee on Growth of the National Academy of Sciences-Research Council is accepting applications for grants-in-aid for cancer research in the United States. Applications received before Oct. 1 will be considered during the winter, and grants recommended at that time become effective on July 1, 1956. *Investigators now receiving support will be notified regarding application for renewal.*

The Committee believes that an understanding of cancer depends upon a deeper insight into the nature of the growth process, normal and malignant. Therefore, the scope of the research program is broad and includes, in addition to clinical investigations on cancer, fundamental studies in the fields of cellular physiology, morphogenesis, genetics, virology, biochemistry, metabolism, nutrition, cytochemistry, physics, radiobiology, chemotherapy, endocrinology, and carcinogenesis. The Committee is particularly interested in encouraging research in the epidemiology of cancer.

Application blanks may be obtained from the Executive Secretary, Committee on Growth, National Research Council, 2101 Constitution Avenue, N. W., Washington 25, D. C.

Books

Biochemistry of Cancer. By Jesse P. Greenstein. Second Edition. Price, $12.00. Pp. 653, with 65 figures and 177 tables. Academic Press, Inc., 125 E. 23rd St., New York 10, 1954.

In the introduction to this book the author remarks that although chemistry is an old art it has not been applied vigorously to the field of cancer research until recently, as medical scientists are beginning to seek a more quantitative approach to the problems of pathology.

The information collected from over 2200 references has been divided into sections dealing with (1) the induction of tumors, (2) attempts at control of induction and growth, and (3) the biochemical properties of the tumor and host tissues. In reading the first section one may well feel that a great amount of work has been done in studying the dosage and response as well as synergism and antagonism shown in the induction of tumors by the many chemicals classified as polycyclic hydrocarbons, azo dyes, halogenated aliphatic hydrocarbons, and amino compounds. Included in this section also is the influence of radiation and environment in initiating neoplasia.

The hormonal induction of tumors as well as the causation by viruses comprises the subject matter in the second half of Section One, dealing with intrinsic factors.

Attempts to control the induction and growth of tumors have produced a multitude of ingenious approaches. By restriction or alteration of the diet, for example, it was hoped that the environment for tumor growth would become inadequate. A similar goal prompted the use of endocrine inhibitors or surgical removal of the gonads or adrenals. The most hopeful type of biochemical treatment has been the use of chemotherapeutic agents, such as the nitrogen mustards, urethane, antimetabolites, bacterial metabolites, benzene, arsenic, colchicine, podophyllum resin, viruses, and radioactive isotopes.

In the section on the biochemical properties of tumors the book reports many of the studies that have been made in an effort to determine the changes in enzyme systems and components that may be correlated with the preneoplastic and neoplastic condition of tissues. One of the interesting fields of study reported involves comparisons of enzyme concentrations in centrifugally fractionated homogenates of normal, precancerous, and cancer tissue. Electrophoretic analysis also has been used in an attempt to differentiate between the mobilities of soluble proteins taken from normal tissues and those taken from tissues approaching or in the cancerous state. The difficulty in achieving decisive information regarding the events leading to cancer suggests that histochemical methods of analysis may be necessary for the solution of the problem. In view of the fact that tumors are able to synthesize protein at the expense of the host, it is not surprising to find that the most active enzymes present are those concerned with protein metabolism, examples being benzoylarginine amidase and dehydropeptidase. At the lower end of the scale of enzyme activity are catalase, cytochrome oxidase, phosphoric and fatty acid esterases, and the cysteine-metabolizing enzymes.

The discussion of the tumor-bearing host has been divided into a consideration of the changes in enzyme concentrations or other components of the blood and tissues, as well as qualitative and quantitative alterations of the urine resulting from liberation of inhibitors by the tumor or the removal of metabolites essential for the normal function of tissues away from the tumor site.

Among the interesting observations of protein metabolism of the tumor-bearing animal is the fact that not only do tumors trap ingested nitrogen but also they make no contribution of nitrogen to the host undergoing a period of starvation.

Another striking effect on the host is the depression of liver catalase activity seen during growth of a tumor followed by a return to normal after surgical removal of the neoplasm. Investigation has revealed that an inhibitor is released by the tumor which brings about this effect in the liver with a drop to one-twentieth of the normal catalase value. At the same time kidney catalase will drop to one-half normal, while the catalase of erythrocytes does not change. This effect seems to be general for most tumor types and is quantitatively related to the increase in the number of tumor cells.

The ready availability of blood for diagnosis should make it ideal for the detection of changes accompanying the growth or induction of cancer, yet no test has been found that is truly specific for cancer and nearly all of the phenomena observed could also result from debilitating diseases.

The high acid phosphatase activity of human prostatic tissue has been observed also in tumors of prostatic epithelium and in metastases in distant parts of the skeleton. The immature prostate has very low levels of this enzyme, which rise with the injection of testosterone propionate or the onset of puberty. The striking elevation in serum acid phosphatase level in patients with disseminated prostatic carcinoma has been shown to drop dramatically in many cases after estrogen treatment or castration.

In considering the present status of the problem, the author states that "A successful and practical control of cancer by systemic means must be applied to those mechanisms which are common and not different in all tumors. There is one phenomenon which appears to be universally common to all tumors, and that is the property of increased autonomy, or the capacity for unlimited or uncontrolled growth. The ultimate control of cancer necessitates an understanding of the chemical factors involved in this capacity, factors presumably absent or present to different degrees in normal tissues." He further asserts that "it is necessary to assume that cancer is a phenomenon coexistent with the living process, that it will be present for some time to come, and that emphasis must be laid on a direct study on the site of malignancy itself."

Über hormonale und morphologische Malignität bei Nebennierengeschwülsten. By Friedrich Stein. Price, not given. Pp. 75, with 28 illustrations. Gustav Fischer, Stuttgart, Germany, 1954.

This monograph consists of detailed case presentations of a functional adrenocortical tumor and a sympathoblastoma, with summaries of 20 cases of adrenocortical tumors. There is a discussion of neurohormonal interrelationships and interactions. The author considers some of the imbalances which may be contributory in the genesis of these tumors. He points out the difficulties present in a clinical classification and comments on the lack of correlation of symptoms with histological findings.

Pathology for the Surgeon. By William Boyd. Price, $12.50. Pp. 737, with 547 illustrations, including 10 in color. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5, 1955.

This seventh edition of Boyd's *Pathology for the Surgeon* follows the sixth edition by eight years. It is up to date, large portions being entirely rewritten and the remainder extensively revised. It is directed primarily to the clinical surgeon in an effort to interpret and elucidate the diseases encountered in surgical practice through pathology. Although there are adequate descriptions of the gross and microscopic lesions involved, the emphasis is on their clinical correlations rather than on fine points of morphological diagnosis. This leads to fairly detailed consideration of physiological alterations underlying morphological changes, as illustrated by the section on Shock. The first eight chapters cover general topics such as inflammation and repair, wound infections, gangrene, shock, and thrombosis; the remaining twenty-six cover the special pathology of the various systems. The writing has the pleasant readability and cultured perspective with which those acquainted with Boyd's earlier texts are familiar. The list of critically selected references on specific topics at the end of each chapter is of great value for more exhaustive study. The printing and illustrations, particularly the photomicrographs, are excellent. This is a well-organized book, fully abreast of more recent contributions to surgical pathology. Because it is so comprehensive in scope, its consideration of some subjects is necessarily cursory. It will prove an excellent text for the student and a useful reference and point of departure for the surgeon and pathologist.

Lucha contra el cáncer: Cincuenta años de mortalidad y morbilidad cancerosa en España. By Prof. Dr. Antonio Llombart and Dr. Ubaldo Gastaminza. Pp. 171. Instituto radio quirúrgico de Guipúzcoa, Industria grafica Valverde, S.A., San Sebastián, Spain. 1954.

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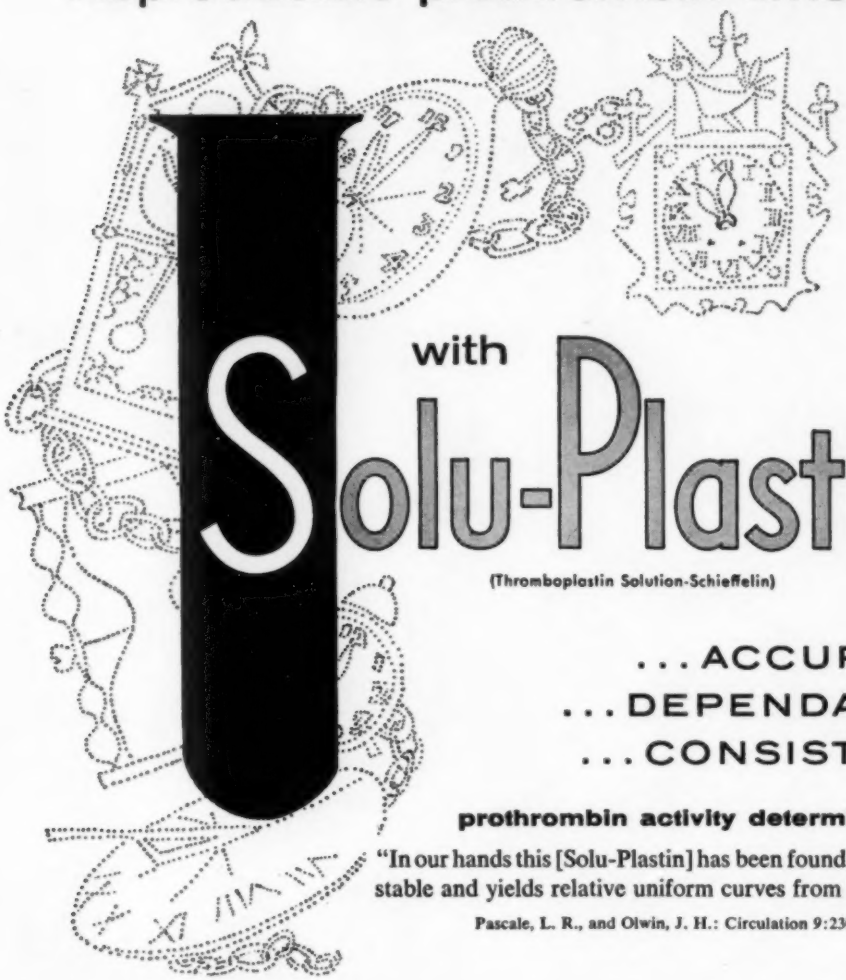

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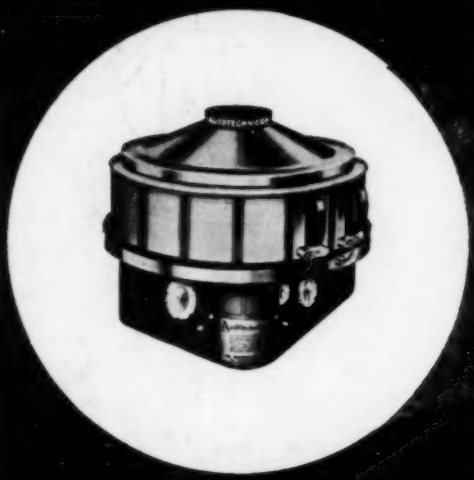

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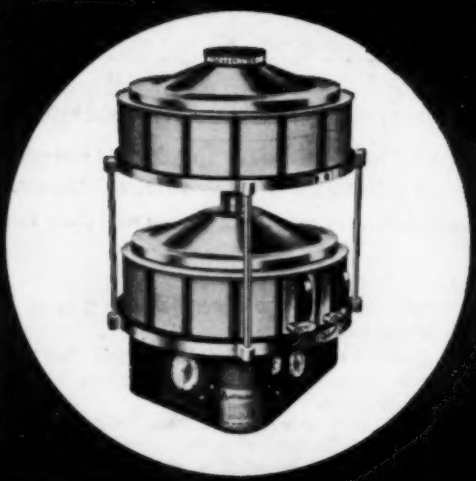

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